PROCEEDINGS
OF THE
SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE

VOL. 30. FEBRUARY, 1933. NO. 5.

6574

Simple Enterostomy Versus Enterostomy Plus Intestinal Evacuation in Ileus.*

ALTON OCHSNER AND AMBROSE STORCK.

From the Department of Surgery, Tulane University School of Medicine.

Whereas the importance of the early relief of mechanical ileus has been appreciated by all clinicians, there has been a controversy concerning whether only a simple relief should be performed or whether in addition to relieving the obstruction the bowel should be emptied of its contents mechanically by "stripping" the intestine. Even though, as shown by experimental and clinical investigations,[1, 2, 3] such a procedure produces marked trauma resulting in cardiovascular changes, the theoretical advantages of complete emptying of the intestine by mechanical "stripping" consist of decompressing the intestine and relieving the strangulation of the intramural intestinal vessels.

In the present investigation the results obtained by simple relief of mechanical ileus were compared with those in which, in addition to the relief of the obstruction, a mechanical evacuation of the intraintestinal contents was secured. Mechanical ileus was produced in dogs and after varying periods of time (from 48 hours to 144 hours), the animal was relaparotomized under sterile precautions. In one series of animals the mechanical obstruction was relieved, whereas in a similar series in addition to the relief of the mechanical obstruction, the dilated loops of intestine were emptied of their

[1] Laewen, A., *Zentralbl. f. Chir.*, 1927, **54**, 1037.

[2] Storck, Ambrose, and Ochsner, Alton, unpublished.

[3] Morton, J. J., *Ann. Surg.*, 1932, **95**, 856.

contents by introducing a catheter into the bowel, and "stripping" the intestine toward the enterostomy tube. The catheter was removed and the opening in the gut sutured. The abdominal wall was closed. After 24 hours, observations were made concerning the activity of the intestine. The animals were laparotomized, balloons were introduced into the lumen of the gut and kymographic tracings obtained as described previously.[4] To determine the intestinal activity, "hypertonic" Hartmann's solution, which previously had been shown to exert a marked stimulating effect on intestine,[5] was administered intravenously to each animal and the intestinal activity recorded. In all, 37 animals were used, of which 12 were eliminated because they did not live long enough to complete the experiment. Seventy-nine observations were made on the 25 remaining animals, 36 following simple relief of obstruction and 43 following relief of obstruction plus mechanical evacuation of the intestinal contents. Four observations were made with 48-hour obstruction, 18 with 72-hour obstruction, 34 with 96-hour obstruction, 17 with 120-hour obstruction, and 6 with 144-hour obstruction. The results obtained following the intravenous administration of 10 cc. of "hypertonic" Hartmann's solution were briefly as follows: Of 36 observations made on intestines in which there had been a simple relief of obstruction, there were increaseses in activity in 31 (86.1%), and no change in activity in 5 (13.8%); of the 43 observations in which in addition to relief of the obstruction the intestine was "stripped", there were increases in activity in 35 (83.7%), no change in 6 (13.9%), and an actual decrease in 2 (4.6%). The average increases in intestinal tone were 15.5 mm., and 10.2 mm., respectively. The average increases in the amplitude of intestinal movement were 1.2 mm. and 0.9 mm., respectively. The average duration of increased activity was 22.9 minutes and 16.7 minutes respectively.

[4] Ochsner, Alton; Gage, I. M., and Cutting, R. A., *Arch. Surg.*, 1930, **21**, 924.

[5] Ochsner, Alton, Gage, I. M., and Cutting, R. A., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 911.

6575

Experimental and Anatomical Studies on the Vagus Nerve of the Cat.

J. O. FOLEY AND F. S. DU BOIS. (Introduced by A. D. Keller.)

From the Department of Anatomy, University of Alabama.

Since Ranson and his associates[1, 2] have demonstrated that the rootlets of the vagus nerve of the dog and the cat are of 2 anatomical types, one resembling the ventral, the other the dorsal roots of thoracic spinal nerves, it seemed desirable to ascertain whether a like differentiation exists in their intramedullary courses. The rootlets of the right vagus nerve were sectioned intracranially in 2 cats and the right nodose ganglion removed in 5 others. After 14 days each animal was killed and the brain stem and cervical cord were fixed in Müller's fluid. Later they were stained by the method of Marchi. Nearly complete series of sections of the pons and medulla oblongata were mounted.*

In such histologic preparations there are 3 types of rootlets. The majority of the degenerated myelinated fibers with cell bodies in the jugular and nodose ganglia enter the medulla by way of the upper (cephalic) rootlets, pass over or through the dorsal portion of the descending trigeminal tract and nucleus, then course dorsal to the motor fascicles of the vagus, and ultimately enter the lateral or ventral portion of the solitary bundle. Probably these coarse fascicles (accompanied by no discernible undegenerated fibers) represent an intramedullary continuation of the pure sensory type seen peripherally by Ranson. Medium sized motor bundles can be traced from the dorsal motor nucleus of the vagus, ventral to the tractus solitarius, through the middle or ventral portion of the spinal nucleus of the trigeminal nerve to the surface of the medulla. Many of these motor fibers proceed peripherally through the lower (caudal) group of rootlets. Finally, coursing with certain motor fascicles are smaller groups of degenerated fibers which leave the efferent bundles in a situation ventral to the solitary tract, turn abruptly dorsalward and enter the latter. These degenerated filaments enter the medulla in company with the upper (cephalic) motor bundles. This combi-

[1] Chase, M. R., and Ranson, S. W., *J. Comp. Neur.*, 1914, **24**, 31.

[2] Ranson, S. W., and Mihalik, P., *Anat. Rec.*, 1932, **54**, 355.

* The authors are indebted to Dr. S. W. Ranson for proposing this problem and for loaning the two series in which the rootlets of the vagus had been sectioned intracranially.

nation of motor and sensory fibers may represent a central continuation of extramedullary mixed rootlets. Some support for this view is obtained from the description by Chase and Ranson of a mixed type of rootlet in the peripheral vagus of the dog.

In view of Ranson's[3] recent demonstration that the sensory rootlets of the first 3 cervical nerves contribute many fibers to the nucleus intermedius of the cat, and in view of DuBois'[4] description of degenerated fibers of the vagus terminating in the nucleus intercalatus of the opossum, these 2 nuclei were carefully studied. In both types of preparations (intracranial section and nodose ganglionectomy) the majority of the myelinated sensory fibers of the vagus nerve terminate in the nuclei of the solitary tract and the commissural nucleus. None were observed to enter either the nucleus intermedius or nucleus intercalatus. In this respect our findings in the cat are in accord with those of Van Gehuchten[5] in the rabbit and Allen[6] in the guinea pig.

6576

Rôle of the Thyroid and of Iodine in Nutritional Anemia.

H. S. MAYERSON.

From the Laboratory of Physiology, School of Medicine, Tulane University of Louisiana.

The experiments here described were undertaken to ascertain whether different functional levels of the thyroid gland as induced by the administration of iodine play any part in the development or severity of the anemia produced by milk feeding.

Kunde, Green and Buno[1] have shown that hypothyroidism is accompanied by mild anemia and Drennan, Malcolm and Cox[2] produced thyroid hyperplasia in rats on a diet of white bread and fresh milk. This hyperplasia was easily corrected by the addition of a small amount of iodine. The iodine content of milk is low but

[3] Ranson, S. W., Davenport, H. K., and Doles, E. A., *J. Comp. Neur.*, 1932, **54**, 1.

[4] DuBois, F. S., *J. Comp. Neur.*, 1929, **47**, 189.

[5] Van Gehuchten, A., *Le Nevraxe.*, 1900, **1**, 173.

[6] Allen, W. F., *J. Comp. Neur.*, 1923, **35**, 171.

[1] Kunde, M. M., Green, M. F., and Buno, G., *Am. J. Physiol.*, 1932, **99**, 469.

[2] Drennan, A. M., Malcolm, J., and Cox, G. A., *Brit. J. Exp. Path.*, 1931, **12**, 430.

varies considerably in different samples,[3] thus suggesting that lack of iodine and consequent thyroid hypofunction may complicate the nutritional anemia in some instances.

Male rats, chosen from litters made anemic by the method of Elvehjem and Kemmerer,[4] were weaned and continued on whole milk powder (Klim) until 6 weeks of age. They were then placed in individual screen bottom cages and for the succeeding 8 weeks were fed the various supplements indicated in the accompanying chart. /The group designated as positive controls represents rats

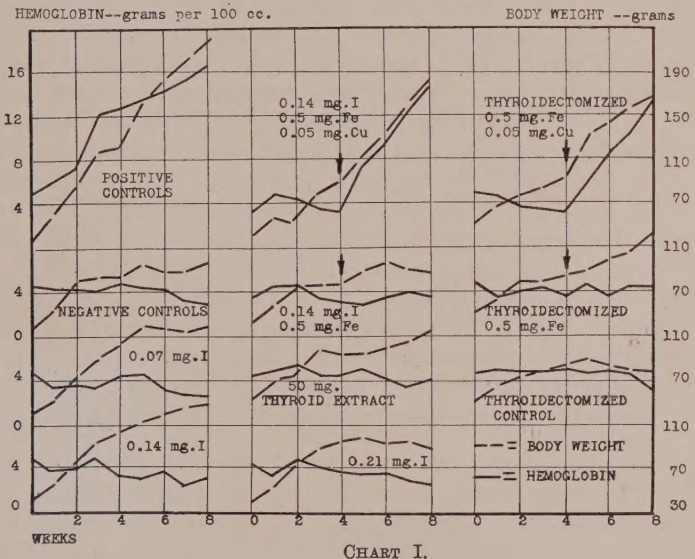


CHART I.

fed the stock diet (Bal-Ra). The negative controls received whole milk powder only, in which no iodine could be detected by the standard methods used.[5] Iodine was given in the form of KI, the unit dose of 0.07 mg. daily being that found by Drennan, Malcolm and Cox[2] to produce (in rats on a diet of white bread and fresh milk) small, colloid glands with a rich iodine content. The amount of thyroid extract fed contained about 0.15 mg. of iodine and represents the maximum amount tolerated without loss of weight. Two groups were given 0.14 mg. iodine daily for 4 weeks, at which time

[3] Krauss, W. E., and Moore, C. F., *J. Biol. Chem.*, 1930, **89**, 581.

[4] Elvehjem, C. A., and Kemmerer, A. R., *J. Biol. Chem.*, 1931, **93**, 189.

[5] Kendall, E. C., *J. Biol. Chem.*, 1914, **19**, 251. Leland, J. P., and Foster, G. L., *J. Biol. Chem.*, 1932, **95**, 165.

the diet of one group was further supplemented with 0.5 mg. iron (as $FeCl_3$) and that of the other group with 0.5 mg. iron plus 0.05 mg. copper (as $CuSO_4$). Fifteen of the rats were thyroidectomized at the age of 3 weeks and continued on the milk diet. At the end of 7 weeks, the diet of 5 animals was supplemented with iron, and that of 5 others with iron and copper, the remaining 5 serving as thyroidectomized controls. Water distilled from glass was used throughout and rigid precautions observed to prevent metallic contamination. Hemoglobin was determined weekly in tail blood with a Newcomer hemoglobinometer, calibrated by oxygen capacity determinations in the Van Slyke and Neill manometric apparatus.

The curves given in the chart are the average values of at least 4 animals in each group. They indicate that various functional levels of the thyroid as induced by the addition of iodine in organic or inorganic combination, and even the complete absence of the thyroid, in no way modify the development or severity of the anemia produced by milk feeding or the hematopoietic response to iron and copper. The results with iron and iodine alone and in combination with copper further substantiate the specific stimulating effect of copper demonstrated by Hart and his co-workers.[6]

Sections of the thyroid glands made at the conclusion of the experiments failed to show any significant abnormalities. Slight hyperplasia was present in several of the negative controls, which, however, in no case exceeded that commonly found in supposedly "normal" animals.

6577

Relation Between Toxicity, Resistance, and Time of Survival.*

L. REINER. (Introduced by C. S. Leonard.)

From the Burroughs Wellcome and Co. Experimental Research Laboratories, Tuckahoe, N. Y.

With collective phenomena it has been often observed that the curve describing the frequency of the occurrence of an event is asymmetrical and not symmetrical as should be expected if the occurrence of the event depended merely on chance. (Gauss's proba-

[6] Hart, E. B., Steenbock, H., Waddell, J., and Elvehjem, C. A., *J. Biol. Chem.*, 1928, **77**, 797.

* Thanks are due to Professor E. B. Wilson for reading critically the manuscript.

bility curve.) This has been generally ascribed to the asymmetrical variation of the parameter on which the occurrence of the event depends. A different explanation is given below. It is shown that asymmetry is to be expected whenever the time which registers the event is a non-linear function of the parameter on which the occurrence of the event depends even though the variation of the same follows the probability rule.

The parameter ξ will be a non-linear function of the time if the event is the consequence of the upset of an equilibrium, *i. e.*, the equilibrium between an existing noxious power (for example, the toxic action of a chemical) and resistance, which upset may result in destruction (for example, death of an organism) which is the registered event. If x and y are the parameters which determine the equilibrium, then the latter will be characterized by

$$\xi = \phi(x) - \psi(y) = 0; \quad t = \infty.$$

Hence

$$t = f\left(\frac{1}{\xi}\right) = f\left[\frac{1}{\phi(x) - \psi(y)}\right].$$

The simplest assumption about f is that any small increase of t will be proportional to the relative decrease of ξ. Thus

$$dt = -a\frac{d\xi}{\xi} = -a\frac{d[\phi(x) - \psi(y)]}{\phi(x) - \psi(y)} \qquad 1$$

and by integration

$$t = -a \ln \xi + K = -a \ln [\phi(x) - \psi(y)] + K. \qquad 2$$

The validity of this equation was tested on the mortality curves of bacteria exposed to a disinfecting agent (disinfection rate curves). These curves very often show a remarkable asymmetry, which is so great that the curves were considered by some to be of the type characteristic of the course of a monomolecular chemical reaction (Madsen and Nyman,[1] Chick,[2] Arrhenius,[3] and others). Eijkman,[4] Hewlett,[5] Reichenbach,[6] Brooks,[7] and others have attributed the asymmetry to a peculiar distribution of resistance among bacteria. From the experiments of Smith[8] and of Loeb and Nor-

[1] Madsen and Nyman, *Z. f. Hyg.*, 1907, **57**, 388.
[2] Chick, *J. Hygiene*, 1908, **8**, 92; 1910, **10**, 237.
[3] Arrhenius, "Quantitative Laws in Biology," 1915.
[4] Eijkman, *Biochem. Z.*, 1908, **11**, 12.
[5] Hewlett, *Lancet*, 1909, March 13, 20, 27.
[6] Reichenbach, *Z. f. Hyg.*, 1911, **69**, 171.
[7] Brooks, *J. Gen. Physiol.*, 1918, **1**, 61.
[8] Smith, *Ann. Appl. Biol.*, 1921, **8**, 27.

throp[9] among others, one notices, however, that the asymmetrical frequency curve changes into a practically symmetrical one for the same culture of organisms if the disinfecting power is increased. Thus it is obvious that the asymmetry cannot be due to a peculiar distribution of resistance in such a case.

It was found that if in equation (1) one puts $\phi(x) = h$ for the constant disinfecting power and $\psi(y) = r$ for the resistance and allows the latter to vary according to the probability rule, symmetrical curves are obtained if $h \gg r$ and asymmetrical curves if h and r are of the same order. Furthermore, if equation (2) was solved for r† and experimental values plotted against $r/(h - r_{med}) = r'$, symmetrical curves were obtained. Applying the Gauss law on the variation of the resistance the experimental curves were recalculated with satisfactory agreement.

It is obvious that the validity of this theory cannot depend on the object of observation, hence it must be applicable to toxicity determinations and to epidemiological phenomena as well as to some analogous processes of non-biological nature. The common feature of these processes is that if the noxious power is sufficiently low a part of the group will escape the occurrence of the event. According to the theory this will occur in spite of the fact that the distribution of the resistance obeys the law of probability. Further consequences of the theory along with details of its proof will be published elsewhere.

---

[9] Loeb and Northrop, *J. Biol. Chem.*, 1917, **32**, 103.

† This was done as follows: Integrating between t_{med} and t_n one obtains $t_{med} - t_n = a \ln \xi_n/\xi_{med}$; a was calculated from values for the median at which point $\xi_o/\xi_{med} = 2$ if the curve is symmetrical. Hence, $a = t_{med}/\ln 2$. Finally resistance intervals were obtained in the usual way:

$$\frac{\xi_n + \xi_{n+1}}{2\,\xi_{med}} = \frac{e^{\frac{t_{med} - t_n}{a}} + e^{\frac{t_{med} - t_{n+1}}{a}}}{2} = \frac{2h - (r_n + r_{n+1})}{2(h - r_{med})} = \text{const.} - \frac{r'_n + r'_{n+1}}{2}$$

From experiments by Smith[8] the average resistance (r_{med}) of *botrytis* spores against phenol was calculated and found to be proportional to the concentration throughout the range for which experimental data were available (0.4—0.7%). Further calculations made since on the action of phenol on anthrax spores (2) showed that in case of a wider range of concentration, the disinfecting power is proportional to the amount of disinfectant adsorbed.

6578

Non Acid-Fast Rods and Granules in Vertical Sections of Mycobacterium Tuberculosis Colonies.

MORTON C. KAHN AND JOSÉ F. NONIDEZ.

From the Departments of Public Health and Anatomy, Cornell University Medical College.

The results of an investigation on the development process of the human *Mycobacterium tuberculosis,* using the single cell method of approach, were reported.[1] Single organisms or small groups of from 2 to 6 were isolated in micro-droplets of Long's medium. These micro-droplets were preserved in such a manner that periodic observations could be made on identical individuals for several days or weeks. While variation did occur, the most usual type of reproductive process was the segmenting of the rod into 3 or more ovoid units, the reduction of these units into fine granules from which, later, extremely fine and delicate rods were found to develop. These rods elongated and thickened with varying rapidity until they became of the size and shape of what may be called the mature acid-fast tubercle bacillus. In the fine granule phase of the development process, as well as in the case of the exceedingly small and delicate rods which were observed to evolve from the granules, the organisms appear distinctly non-acid-fast.

The changes which were described are seemingly not allied to those recently described by Mellon and his coworkers[2] for the avian *M. tuberculosis,* in which a filterable phase is involved. In a recent study of one of us (K) no positive results with human *M. tuberculosis,* as far as filterability is concerned, have been obtained by culture or animal inoculation when the N, V, or W Berkefeld candles are employed, or with the neutrally charged membrane filters of Zsigmondy-Bachman. An occasional filtrate was found to become clouded with coccoid and diplococcoid types after long incubation, but upon critical examination the organism which grew up was found to be a non-pathogenic member of the diphtheroid group, which at times produces a considerable amount of acid-fast material and therefore could possibly be mistaken for *Mycobacterium tuberculosis.* An organism having the identical characteristics of this diphtheroid has purposely been isolated from the air. Another point

[1] Kahn, *Am. Rev. Tuberculosis,* 1929, **20**, 150.

[2] Mellon, Richardson and Fisher, *J. Bact.,* 1933, **25**, 45.

of departure from the work reported by Mellon and his coworkers lies in the fact that the morphological changes which we described took place in the identical microdroplet of medium in which the original single cell was isolated. Transfers to different types of media were not found necessary, and accordingly any possibility of contamination from the air was avoided. Nor was it possible to fix the organism in any one of the morphological stages. Changes, when they did occur, were invariably found to be progressive in nature.

Oerskov[3] and Bergel[4] have considered the non-acid-fast rods and granules to be degenerative forms of the *M. tuberculosis,* appearing in greatest number in old cultures. One of us (K) has not been able to substantiate this claim upon examining cultures of from 10 days to 6 months of age. It is true, however, that the non-acid-fast rods and granules are not readily recognized by those unfamiliar with the above mentioned development process, when the usual Ziehl-Neelsen staining method is applied to direct smears obtained from culture on liquid or solid medium. The following technic has been successfully applied by one of us (N) to the making of vertical sections of colonies grown on egg medium and also to growth membrane developed upon the liquid medium of Long. Upon examination these sections revealed large numbers of unmistakably non-acid-fast rods and granules which, as will be described, occur in such a position as to strongly suggest their being the youngest forms of *M. tuberculosis.*

Small blocks of the medium with the attached colonies were fixed in 95% alcohol for 24 hours, dehydrated with absolute alcohol and cleared with xylol. They were embedded in paraffin and cut into vertical sections 4 micra in thickness. The sections were fixed to the slide with a minimum amount of albumen glycerin mixture. The paraffin was dissolved with xylol, the sections rinsed once more with pure xylol and flooded with absolute alcohol to remove any trace of the paraffin solvent. They were stained with the Ziehl-Neelsen method and after drying were covered with a drop of damar and a cover glass added. The same procedure was followed for the study of the growth membrane taken from liquid medium, but the latter was found to be crumbly and had to be handled very carefully to prevent its disintegration.

In order to meet possible objections to the action of such fat solvents as alcohol and xylol on the organisms, the procedure used

[3] Oerskov, *Z. fur Bakt. Parasit. u. Infekt.,* 1932, **123,** 271.

[4] Bergel, *Z. Tbk.,* 1914, **22,** 343.

for the preservation of fats and lipoids was also applied. Colonies attached to the egg medium were fixed in 15% neutral formalin for 48 hours. They were then suspended in a 3% solution of warm agar. After cooling, a block was trimmed. The block of agar enclosing the colony was placed upon a drop of distilled water on the stage of the freezing microtome and frozen with liquid CO_2. Vertical sections of the colony were cut. The sections were lifted from the edge of the knife with a fine brush and put into sterile distilled water, where they spread. They were transferred to slides smeared with albumen fixative, spread flat, allowed to dry, and stained with the Ziehl-Neelsen method in the usual way, finally being mounted in damar under a cover glass. The about to be described non-acid-fast rods and granules appeared in both the colonies treated with alcohol and xylol, and in the sections of colonies cut with the freezing microtome on which no fat solvent was used. The presence of these non-acid-fast forms cannot, therefore, be attributed to the uneven dissolution of the fatty substance by the reagents mentioned above. If alcohol and xylol do exercise any influence on the staining capacity of *M. tuberculosis*, the latter would seldom have been seen in its acid-fast condition in sections of tuberculous tissue when prepared with the ordinary histo-pathological methods, for the organisms occur in small numbers and usually scattered. Unless the penetration by xylol be thorough, it would be impossible to cut sections of such material. As a further control, however, a number of thin smears were made from a growing colony of *M. tuberculosis*. Some of the slides were stained with the Ziehl-Neelsen method as controls, while the others were placed in a mixture of equal parts of absolute alcohol and xylol for 24 hours. They were thoroughly rinsed with absolute alcohol, allowed to dry, and then stained with the aforementioned technic. No relative increase of non-acid-fast to acid-fast organisms occurred. In other words, though the mycobacteria were thinly scattered over the slide, 24 hours in these reagents did not influence their subsequent staining. A similar experiment was performed with tuberculous sputum some years ago by Dr. Ralph Stillman of this institution, also with negative results.[5]

Finally, a word must be said for the use of albumen for fixing sections to the slide. Albumen fixative is a mixture of equal parts of the whites of fresh eggs and glycerin. It is carefully filtered after preparation. When spread thin upon a clean slide, it does not take the stains, hence its current use in histology. In order to make sure that no background could result from the use of this mixture in any

[5] Personal communication.

way simulating micro-organisms, and that the paraffin used for embedding did not leave a confusing residue, paraffin sections not containing the colony were fixed to slides smeared with the albumen glycerin. After the paraffin was dissolved with xylol and the slide rinsed with absolute alcohol, the staining method of Ziehl-Neelsen was applied. Nothing resembling rods or even granules could be seen in these preparations, the background being entirely colorless.

Upon examination of a vertical section of a human or bovine *M. tuberculosis* colony some 6 weeks of age, which has been stained with the Ziehl-Neelsen method, the low power of the microscope will reveal 3 distinctly stratified zones. The lower zone, in closest contact with the medium at its base, extends up through the colony some two-thirds to four-fifths of its entire depth and appears strongly acid-fast. The remaining peripheral zone may be divided into 2 parts, the outermost of which appears definitely non-acid-fast. Directly under this there is a very narrow but distinct area which appears as a stratum of indefinite pale red color, considerably less strongly acid-fast than the lower area above mentioned. When an examination is made with the oil immersion magnification, the outer peripheral zone is found to be composed of myriads of non-acid-fast rods and granules, as well as acid-fast rods containing the dark appearing intracellular areas. The lower stratum, which is strongly acid-fast, is composed principally of large, strongly acid-fast rods, with a few non-acid-fast rods and granules scattered through the cracks and crevices which appear in this portion of the colony. The lighter staining central stratum is composed principally of pale acid-fast rods and granules, although relatively a considerable number of non-acid-fast forms are also encountered in this locality.

The question at once suggests itself as to which is the older portion of the colony, the peripheral zone or the lowest stratum. If the peripheral zone were the oldest, then the claims of Oerskov—who believes the non-acid-fast rods and granules to be old degenerative forms—may well be correct. We, however, believe that the reverse is true, and that the peripheral zone containing the myriads of non-acid-fast rods and granules is the youngest portion of the colony and the zone of active growth. Our reasons are: 1. When vertical sections are made through old colonies of *M. tuberculosis* which have ceased their growth, the peripheral zone is almost entirely obliterated, there being only a few non-acid-fast forms scattered here and there at the extreme outer edge of the colony, while the remaining areas appear strongly acid-fast. 2. Vertical sec-

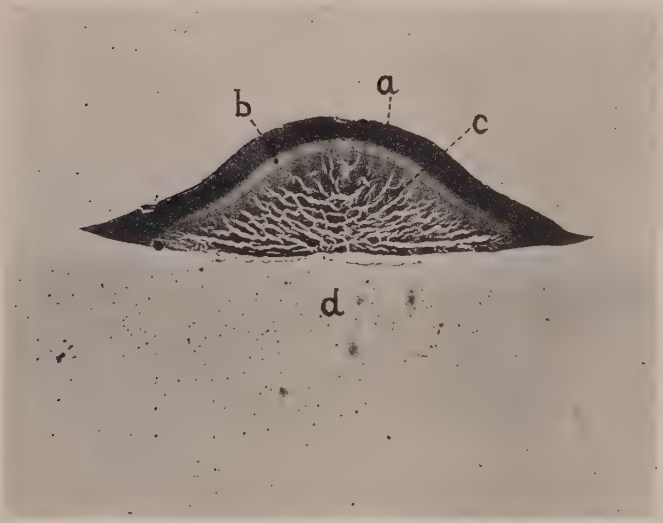


FIG. 1.

PHOTO-MICROGRAPH OF STAINED VERTICAL SECTION OF MYCOBACTERIUM TUBERCULOSIS COLONY. ×200.

The peripheral zone A contains myriads of non-acid-fast rods and non-acid-fast granules, as well as young acid-fast rods which contain the dark staining intracellular elements. Zone B contains the lighter staining but definitely acid-fast rods and granules. Zone C is composed chiefly of large, strongly acid-fast rods, with a few non-acid-fast rods and granules occurring in the cracks and crevices. Zone D, egg medium. The space between the colony and the egg medium is of course due to shrinkage. The cracking of the acid-fast core of the colony in this case is an artefact due to the drying of the sections prior to Ziehl-Neelsen staining. However, when silver nitrate is used for impregnation and the sections are not allowed to dry, a number of well defined vacuoles are present in this strongly acid-fast "medulla", which give additional evidence that this portion of the colony is the oldest, for these vacuoles do not appear in the peripheral zone.

tions have also been made through young and old growth membrane taken from Long's liquid medium. The young, thin, white growth membrane reveals myriads of non-acid-fast rods and granules, with the beaded acid-fast forms scattered here and there through the mass, while the yellow, crusty, folded, older area of growth membrane gives rise to relatively few of the non-acid-fast types. 3. The mycobacteria at the periphery of the colony are presumably receiving the most oxygen and the exact oxygen requirements of this organism are well known. 4. The single cell studies on the development process reported by one of us (K) indicated the non-acid-fast rods and granules to be the youngest form of *M. tuberculosis,* and also that the acid-fast rods with the dark intracellular elements were

the first step in the formation of a new generation. The information obtained from a study of the vertical sections of both human and bovine colonies of *M. tuberculosis* would seem to substantiate this claim.

It seems as though we must conceive of the colony as a half sphere and presumably some growth is taking place throughout the entire area as evidenced by the few non-acid-fast rods and granules which may be seen scattered even through the strongly acid-fast "medulla". The peripheral zone, however, reveals considerably larger numbers of these forms, and is therefore presumably the area in which most active growth is taking place.

6579

Precipitins Against Fractions of Streptococci in Hemolytic Streptococcus Disease, Glomerular Nephritis, Rheumatoid Arthritis, and S. Viridans Endocarditis.

DAVID SEEGAL, MICHAEL HEIDELBERGER, ELIZABETH L. JOST AND JOHN D. LYTTLE.

From the Department of Medicine, College of Physicians and Surgeons, Columbia University, the Presbyterian Hospital, and the Department of Pediatrics, Babies Hospital, New York City.

The sera of 310 patients have been studied for precipitins against 2 protein fractions of *S. hemolyticus*, the nucleoprotein of *S. viridans* and the group specific carbohydrate of *S. hemolyticus*. The *S. hemolyticus* fractions were freshly prepared. The *S. viridans* protein first used* was 5 years old. Later tests with a sample newly isolated from the same strain gave identical results. A description of the precipitin test and the antigens used has been presented in a previous communication.[1]

Sera from the following groups of cases have been studied:

1. A control group of 39 healthy nurses during the fall season.†
2. A control group of 16 healthy medical students and nurses during the spring season.

* Kindly supplied by Dr. Rebecca C. Lancefield of the Hospital of the Rockefeller Institute for Medical Research.

[1] Seegal, D., Heidelberger, M., and Jost, E. L., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 939.

† These data were made available by Dr. A. F. Coburn.

3. A control disease group of 17 cases with temperatures of 102° or over. These patients were not affected with hemolytic streptococcus disease. All these sera were obtained during the spring season.

4. A control disease group of 100 cases during the spring season. None of these patients was known to have rheumatic fever, rheumatoid arthritis, sub-acute *S. viridans* endocarditis, proven hemolytic streptococcus disease, glomerular nephritis or peptic ulcer.

5. Twenty-three cases of proven sub-acute *S. viridans* endocarditis.[1]

6. Thirty-six cases of rheumatoid arthritis.‡

7. Sixteen cases of proven hemolytic streptococcus disease.

8. Fourteen cases of acute glomerular nephritis.

9. Sixteen cases of sub-acute glomerular nephritis.

10. Seven cases of healed glomerular nephritis.

11. Five cases of healed glomerular nephritis during hemolytic streptococcus infection.

12. Twenty-one cases of peptic ulcer with symptoms.

A series of tests was made on the sera of each patient with hemolytic streptococcus infection and glomerular nephritis. The precipitin test shown in the table was the maximum value obtained during the 2 to 5 week period after the known onset of the patient's illness. This procedure depended upon our experience that the precipitin test against fractions of the hemolytic streptococcus in frank hemolytic streptococcus disease and glomerular nephritis is negative or very weak in the early days of the disease. Usually 2 to 5 weeks are required for the development of the antibody if it is to appear.

The accompanying table illustrates the variation in the maximum degree of precipitin formation in the groups studied thus far.

The following conclusions may be drawn: 1. Precipitins against the 3 fractions of the *S. hemolyticus* and the one *S. viridans* protein are absent or minimal in the 4 control groups. 2. Strong precipitin formation against the hemolytic streptococcus protein antigens is noted chiefly in the cases of subacute *S. viridans* endocarditis and rheumatoid arthritis. 3. Moderate precipitin formation against these same fractions is noted in many of the cases of proven hemolytic streptococcus disease, acute and subacute glomerular nephritis and peptic ulcer.

4. The strongest reactions against the group specific carbohydrate of *S. hemolyticus* occur in the sera of patients with either proven hemolytic streptococcus infection or rheumatoid arthritis.

‡ These sera were made available by Dr. M. H. Dawson.

TABLE I.
Precipitins in Human Sera Against Fractions of *S. hemolyticus* and *S. viridans*.

Precipitin titer§	Control Fall Group	Control Spring Group	Control Disease Fever Group (age ?? or over)	Control Disease Group	*S. viridans* endocarditis	Rheumatoid Arthritis	*S. hemolyticus* Infection	Acute Glomerular Nephritis	Subacute or Chronic Glomerular Nephritis	Healed Glomerular Nephritis	Healed Glomerular Nephritis during *S. hemolyticus* infection	Peptic Ulcer with Symptoms
				S. Hemolyticus Nucleoprotein D								
++++	[illegible]	[illegible]	[illegible]	0	2	[illegible]	0	0	0	0	0	0
+++	[illegible]	[illegible]	[illegible]	0	9	[illegible]	3	1	2	0	0	2
++	[illegible]	[illegible]	[illegible]	3	8	[illegible]	7	6	4	0	2	5
+	[illegible]	[illegible]	[illegible]	24	3	[illegible]	3	2	3	2	1	6
0	[illegible]	10	15	73	1	[illegible]	3	5	6	5	2	8
Total No. Cases	[illegible]	16	17	100	23	[illegible]	16	14	15	7	5	21
				S. Hemolyticus Nucleoprotein K								
++++	[illegible]	[illegible]	[illegible]	[illegible]	1	[illegible]	1	1	[illegible]	0	0	[illegible]
+++	[illegible]	[illegible]	[illegible]	[illegible]	16	11	3	[illegible]	[illegible]	0	0	[illegible]
++	[illegible]	[illegible]	[illegible]	[illegible]	0	[illegible]	3	[illegible]	[illegible]	0	0	[illegible]
+	[illegible]	[illegible]	[illegible]	[illegible]	2	[illegible]	5	[illegible]	[illegible]	1	0	[illegible]
0	[illegible]	16	17	[illegible]	4	[illegible]	4	[illegible]	[illegible]	5	4	[illegible]
Total No. Cases	39	16	17	[illegible]	[illegible]	[illegible]	16	14	[illegible]	6	4	21
				S. Hemolyticus Carbohydrate C.								
++++		[illegible]	[illegible]	[illegible]	0	1	2	0	[illegible]	0	0	0
+++		[illegible]	[illegible]	[illegible]	2	6	3	1	[illegible]	0	0	1
++		[illegible]	[illegible]	[illegible]	1	5	2	3	[illegible]	0	0	0
+		[illegible]	[illegible]	[illegible]	4	2	0	0	[illegible]	1	2	1
0		16	17	[illegible]	16	[illegible]	9	10	14	5	3	19
Total No. Cases		16	17	[illegible]	23	[illegible]	16	14	[illegible]	6	5	21
				S. Viridans Nucleoprotein								
++++		[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	0	[illegible]
+++		[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	0	[illegible]
++		[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	0	[illegible]
+		[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	0	[illegible]
0		[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	1	[illegible]
Total No. Cases		[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	1	[illegible]

Nucleoprotein D: Acetic acid precipitable *S. hemolyticus* protein extractable at neutrality; concentration, 1:2000.

Nucleoprotein K: Acetic acid [illegible] 1:2000.

Carbohydrate C: Species specific polysaccharide: concentration, 1:200,000.

Concentration of *S. viridans* nucleoprotein: 1:2000.

§ The precipitin titer was [illegible] as in (1). These readings represent the precipitates after standing over night in the cold and centrifuging.

Seven members of the group of 16 cases of proven hemolytic streptococcus infection showed a strong anti-carbohydrate reaction. Four of these 7 had positive blood cultures of hemolytic streptococcus

Only one of the 9 remaining cases with negative anti-carbohydrate reactions had a positive blood culture. This fact is in agreement with our unpublished observations that rabbits injected subcutaneously with heat-killed *S. hemolyticus* only occasionally develop precipitins against the carbohydrate fraction, although the same rabbits generally develop this precipitin when the organism is injected intravenously.

5. The cases of *S. viridans* endocarditis were the only group in which precipitin formation against the *S. viridans* nucleoprotein was marked. It is of interest, in this respect, that the precipitin reactions in the cases of rheumatoid arthritis more closely correspond to those seen in hemolytic streptococcus disease than to those found in *S. viridans* endocarditis.

6. The *S. hemolyticus* protein precipitin tests in the cases of acute and subacute nephritis in this series closely parallel those found in proven hemolytic streptococcus disease. This observation is additional evidence in favor of the opinion that glomerular nephritis may be related to hemolytic streptococcus infection. The 7 cases of healed glomerular nephritis show no significant precipitin formation. In this series, in 5 cases of healed glomerular nephritis a hemolytic streptococcus infection occurred without an exacerbation of the nephritis. Precipitin formation in this group is present, but diminished.

7. The finding of precipitins in the sera of patients with active peptic ulcer is unexplained, and is of interest since Derick and Fulton[2] obtained a high percentage of positive skin reactions in this group with comparable streptococcus protein fractions.

8. Some sera from normal patients and those with streptococcus disease which show no precipitins when tested within a few days may give a moderately positive test after remaining in the ice-box one to 9 weeks. The development of this reaction is in many instances paralleled by a non-specific reaction against a typhoid nucleoprotein. With few exceptions, inactivation of these old sera by heating at 56° C. for one-half hour prevents this reaction.

[2] Derick, C. L., and Fulton, M. M., *J. Clin. Invest.*, 1931, **10**, 121.

6580

Filtration and Secretion of Exogenous Creatinine in Man.

NORMAN JOLLIFFE AND HERBERT CHASIS.

From the Departments of Physiology and Medicine, University and Bellevue Hospital Medical College, New York University.

Jolliffe, Shannon and Smith[1] have presented evidence that the excretion of xylose and other non-metabolized sugars can be used to measure glomerular filtration in the dog, and by simultaneous xylose and creatinine clearances they have demonstrated the tubular secretion of creatinine in that animal. The use of xylose for measuring glomerular filtration has been confirmed by Clark and Smith[2] in the elasmobranch, *Squalus acanthias* and by Marshall[3] in the frog, *Rana catesbiana*.

A comparison of the excretion of xylose and creatinine in man is of particular interest, since the latter substance has been widely used on Rehberg's[4] recommendation as a measure of glomerular filtrate.

The subjects of this investigation were healthy, male, medical students between the ages of 20 and 30; xylose, creatinine and urea clearances were measured in the morning after no breakfast or a light meal in which milk, tea, coffee and protein were excluded.

Xylose (50 gm.) and creatinine (10 gm.) were administered separately with varying amounts of water 90 and 60 minutes respectively prior to the start of the first urine collection period. Three or more consecutive 20-minute periods were observed; in most of these the rate of urine flow was above the lower figure (1.7) given as the augmentation limit of urea.[5] Blood samples were withdrawn at 30-minute intervals and plasma concentrations were interpolated to the middle of each urine period. Plasma and urine (diluted to the expected U/P ratio) were analyzed by the methods described by Shannon, Jolliffe and Smith.[6]

The average clearances (UV/P.S.A.) expressed as cc. per minute per square meter of body surface are: urea, 35.1; xylose, 51.3, and

[1] Jolliffe, N., Shannon, J. A., and Smith, H. W., *Am. J. Phys.*, 1932, **100**, 301.

[2] Clarke, R. W., and Smith, H. W., *J. Comp. and Cell. Phys.*, 1932, **1**, 131.

[3] Marshall, E. K., Jr., *J. Comp. and Cell. Phys.*, 1932, **2**, 349.

[4] Rehberg, P. B., *Biochem. J.*, 1926, **20**, 447.

[5] Moller, E., McIntosh, J. F., and Van Slyke, D. D., *J. Clin. Invest.*, 1928, **6**, 427.

[6] Shannon, J. A., Jolliffe, N., and Smith, H. W., *Am. J. Phys.*, 1932, **102**, 534.

creatinine, 89.1. The average creatinine/xylose ratio was 1.74, and the average urea xylose ratio was 0.684. The creatinine xylose ratio for man (1.74) is thus somewhat higher than was reported for the dog (1.40). Accepting the xylose clearances as measuring the glomerular filtrate, it appears that a considerable quantity of creatinine is removed from the blood by some mechanism other than glomerular filtration, amounting to about 75% of the filtered creatinine, or 43% of the total creatinine excreted. It is inferred that this moiety is removed from the blood and excreted into the urine by tubular secretion.

6581

Metabolism of d- and l-Methionine.

RICHARD W. JACKSON AND RICHARD J. BLOCK.
(Introduced by Arthur H. Smith.)
From the Department of Physiological Chemistry, Yale University.

Previous investigation of the physiological rôle of methionine in the animal organism led the writers to conclude that "methionine, like cystine, is capable of unmistakably stimulating growth in albino rats subsisting on a basal diet poor in cystine.[1, 2] It was pointed out[2] that this observation immediately raised various questions relative to the intermediary metabolism of methionine. Referring to one of these problems, we stated: "It is obvious, of course, that, since the addition of methionine (*dl*) to the diet of animals subsisting on the regimen previously described leads to growth stimulation, the study of the physiological behavior of . . . . . the separate optically active forms of methionine becomes important." These compounds have been investigated with the following results.

Methionine was synthesized and resolved according to the methods of Windus and Marvel.[3, 4] *d*-Methionine as well as the naturally occurring *l*-methionine stimulates growth in the rat ingesting our cystine-methionine deficient diet. (*cf.*, the results of similar experi-

[1] Jackson, R. W., and Block, R. J., *Science*, 1931, **74**, 414.
[2] Jackson, R. W., and Block, R. J., *J. Biol. Chem.*, 1932, **98**, 465.
[3] Windus, W., and Marvel, C. S., *J. Am. Chem. Soc.*, 1930, **52**, 2575.
[4] Windus, W., and Marvel, C. S., *J. Am. Chem. Soc.*, 1931, **53**, 3490.

ments on tryptophane and cystine.[3,4,5,6,7] The formyl derivatives of the 2 optical isomers of methionine also were tested. The administration of formyl l-methionine causes increments of body weight similar to those produced by both l- and d-methionine. On the other hand, formyl d-methionine apparently cannot be utilized by the animal organism for growth under the conditions of our experiments. Analogous observations have been made on the physiological availability of the acetyl derivatives of d- and l-tryptophane.[8]

6582

Anterior Pituitary and Lactation.

H. SELYE, J. B. COLLIP AND D. L. THOMSON.

From the Department of Biochemistry, McGill University, Montreal.

By injection of the anterior-pituitary-like hormone (A.P.L.) of pregnancy urine Evans and Simpson[1] were successful in producing marked development of the mammary glands in virgin rats, but were unable to produce milk secretion; Bradbury[2] obtained similar results in mice. We have confirmed these results by histological examination, which shows that no secretion takes place in the glands, although the alveoli are as numerous as in late pregnancy. We further observed that the development of the mammary gland in these animals runs parallel to the increase in the weight of their ovaries.

We found, however, that removal of the intensely luteinized ovaries of these rats will lead to abundant milk secretion in their mammary glands within 36 hours (13 experiments, all positive). We further observed that if the pituitary was removed simultaneously with the ovaries, milk secretion did not set in (4 experiments).

These experiments seem to indicate that removal of the luteinized ovaries will lead to milk secretion in the fully developed mammary

[3] Berg, C. P., and Potgieter, M., *J. Biol. Chem.*, 1932, **94**, 661.

[4] du Vigneaud, V., Sealock, R. R., and Van Etten, C., *J. Biol. Chem.*, 1932, **98**, 565.

[5] Lawrie, N. R., *Biochem. J.*, 1932, **26**, 435.

[6] du Vigneaud, V., Dorfmann, R., and Loring, H. S., *J. Biol. Chem.*, 1932, **98**, 577.

[7] Evans, H. M., and Simpson, M. E., *Am. J. Physiol.*, 1931, **98**, 511.

[8] Bradbury, J. T., PROC. SOC. EXP. BIOL. AND MED., 1932, **30**, 212.

FIG. 1. *Milk Secretion.*
Mammary glands were removed from rats and dropped into dilute formalin. On the right is seen a gland from a normal lactating rat, the milk having made the solution turbid. The gland in the center bottle was removed from a virgin rat injected daily with 200-day units of A.P.L., starting when the rat was 27 days old and continuing for 26 days. Although great development has taken place, there is no milk secretion. The gland on the left was taken from a rat similarly treated and then castrated, showing abundant milk production.

gland of the A.P.L. treated rat, but only in the presence of the pituitary.

6583

Effect of Hypophysectomy Upon Pregnancy and Lactation.

H. SELYE, J. B. COLLIP AND D. L. THOMSON.

From the Department of Biochemistry, McGill University, Montreal, Canada.

The considerable enlargement and structural changes of the pituitary during pregnancy and lactation seem to indicate that the internal secretion of this organ plays a very important rôle during this period. Therefore repeated attempts have been made to determine whether pregnancy can be maintained after hypophysectomy, but the results of such experiments are contradictory.

Whereas Aschner[1] found that abortion takes place in hypophysectomized pregnant dogs, Allan and Wiles[2] observed that pregnancy

[1] Aschner, B., *Pflügers Arch. ges Physiol.*, 1912, **146**, 1.

[2] Allan, H., and Wiles, P., *J. Physiol.*, 1932, **75**, 23.

and parturition are not interfered with in the hypophysectomized cat, although lactation is impossible. Pencharz and Long,[3] working with rats, stated that pregnancy is not interrupted by removal of the hypophysis, but the process of parturition becomes impossible and the foetuses die *in utero* after a somewhat prolonged gestation period.

Repeating these experiments on rats we were able to confirm the statement that pregnancy is usually prolonged (up to 26 days). If the pituitary is removed between the tenth and fourteenth day of gestation, death and resorption of the foetuses may occur; but when the pregnancy proceeded normally until term, in 22 out of 24 cases the mechanism of parturition was not interfered with, and the litters were born alive; in the 2 exceptional cases hemorrhage occurred at term and the foetuses died *in utero*. We further established that the milk secretion always sets in normally at birth, but stops after a few hours, so that the hypophysectomized mother is unable to nurse her young.

As has been pointed out previously,[4] milk secretion will also stop immediately if the pituitary is removed in various stages of lactation.

These experiments indicate that the endocrine functions of the pituitary are not indispensable during the second part of pregnancy and parturition in the rat. Milk secretion can also begin in their absence but stops a few hours after the litter has been born.

6584

Effect of Prolonged Administration of the Anterior Pituitary-Like Hormone on Pituitary and Thyroid.

J. B. COLLIP, H. SELYE, D. L. THOMSON AND J. E. WILLIAMSON.

From the Department of Biochemistry, McGill University, Montreal, Canada.

Changes in the anterior lobe of the pituitary after administration of the anterior pituitary-like hormone (A.P.L.) of pregnancy urine or placenta have been observed by numerous investigators,[1, 2, 3] but

[3] Pencharz, R. I., and Long, J. A., *Science*, 1931, **74**, 206.

[4] Collip, J. B., Selye, H., and Thomson, D. L., *Nature*, 1933, **131**, 56.

[1] Baniecki, H., *Arch. f. Gynäkol.*, 1928, **134**, 693.

[2] Zondek, B., and Berblinger, W., *Klin. Wochschr.*, 1931, **10**, 1061.

[3] Zondek, B., *Hormone des Ovariums unde des Hypophysenvorderlappens*, Berlin, 1931.

their results are contradictory, both as regards the histological nature of the changes thus produced and the effectiveness or ineffectiveness of A.P.L. in the male.

Zondek[3] found that when prolan is given to female rats over a long period the ovaries, which have increased in size in the beginning, will become smaller again. These observations have been confirmed in this Department, and it has also been found that the same retrogression in size takes place in the sex organs of the male if A.P.L is given over a very long period.[4]

We thought that this decrease in sensitivity to A.P.L. which sets in gradually in chronic experiments might be at least partly responsible for the discrepancies in the results of those investigators who studied the effect of A.P.L. on the pituitary. Our experiments seem to confirm this view. We found in several series of experiments in which injections were given from the 27th to the 67th day of life, that the size of the pituitary runs parallel to the size of the ovaries in the A.P.L. treated female rat. In 16 rats observed at the time when the enlargement of the ovaries was most conspicuous (0.547-0.616 gm. as compared with 0.032 in the untreated control females of the same age) the pituitary was also at its maximum size (0.0115-0.012 gm. as compared with 0.004-0.0045 gm. in the control animals). This increase in weight is due solely to the enlargement of the anterior lobe. No pituitary enlargement was found in the A.P.L.-treated male rats (11 animals). We found, further, that the thyroid was also very much enlarged (200-300%) and showed histological signs of hyperplasia in the females at the time when their ovaries attained their greatest weight, whereas no thyroid enlargement could be produced in the male. A.P.L. also had no effect on the thyroids of hypophysectomized rats, either male or female.

These experiments indicate that a very definite enlargement of the pituitary and thyroid can be produced in the female rat by prolonged A.P.L. administration; the enlargement of both these organs running approximately parallel to the weight increase of the ovaries.

[4] Collip, J. B., Thomson, D. L., McPhail, M. K., and Williamson, J. E., *Canad. Med. Assn. J.*, 1931, **24**, 201.

6585

The Estrous Cycle and the Adrenal Glands.*

E. L. COREY AND S. W. BRITTON. (Introduced by H. E. Jordan.)

From the Physiological Laboratory, University of Virginia Medical School.

About 2 years ago experiments were reported from this laboratory showing important interrelationships between the adrenal cortex and the gonads. It was definitely shown that very early maturation of the sex glands could be induced by treatment of animals with cortico-adrenal extract.[1] The present brief report mainly indicates the effects of cortico-adrenal extract on the estrous cycle in normal and adrenalectomized rats. The influence of other sex hormones is also touched upon.

We have carried out observations on about 50 animals under various experimental conditions. Vaginal smears have been made daily over periods of several weeks according to the method of Long and Evans.[2] The extract of the adrenal cortex employed was similar to that previously described.[3, 4] The potency of the preparations was proved by its restorative effect when injected into adrenalectomized cats showing insufficiency symptoms. A modified Swingle-Pfiffner method of extraction was used.[5] The chief results may be summarized as follows: Cortico-adrenal extract when injected into normal rats produced a modification of the estrous cycle in the direction of increased activity, the estrous type of smear being often observed for several days continuously. Later, in many cases, an apparent inhibition of estrus was noted.

Removal of the adrenal glands resulted in complete suppression of the estrous cycle in about 90% of cases (in agreement with the observations of Martin[6]).

In adrenalectomized animals in which estrus had been inhibited for various long periods, cortico-adrenal extract restored the cycle to normal, and maintained their lives indefinitely. When extract administration was stopped, in such cases, the estrous cycle was

* Grateful acknowledgment is made of aid received in this investigation from the Committee for Research in Problems of Sex of the National Research Council.

[1] Corey, E. L., and Britton, S. W., *Am. J. Physiol.*, 1931, **99**, 33.

[2] Long, J. A., and Evans, H. M., *Univ. of Cal. Memoirs*, 1922.

[3] Britton, S. W., and Silvette, H., *Am. J. Physiol.*, 1931, **99**, 15.

[4] Britton, S. W., and Silvette, H., *Am. J. Physiol.*, 1932, **100**, 701.

[5] Swingle, W. W., and Pfiffner, J. J., *Am. J. Physiol.*, 1931, **96**, 164.

[6] Martin, S. J., *Am. J. Physiol.*, 1932, **100**, 180.

again completely inhibited, and the animals succumbed with characteristic symptoms.

In several cases in which theelin or antuitrin "S"† was administered following adrenalectomy and inhibition of estrus, the restoration of estrus was brought about by such treatment.

Repeated administration of cortico-adrenal extract to young, immature female rats brought about a slightly earlier opening of the vagina than in litter controls.

6586

Effects of Alternate Suction and Pressure on Circulation in the Lower Extremities.

EUGENE M. LANDIS AND JOHN H. GIBBON, JR.

(Introduced by M. H. Jacobs.)

From the Robinette Foundation, University of Pennsylvania Hospital, Philadelphia.

According to Poiseuille's law the volume of fluid flowing through a rigid tube is proportional to the fall in pressure along the tube. It seems possible, therefore, that if the peripheral fall in blood pressure could be increased the total amount of blood flowing past an arterial obstruction in unit time would be greater. The fall in pressure through the arterial tree may be increased physically in two ways, (a) by elevating aortic blood pressure or (b) by reducing peripheral blood pressure to a negative value. The first method is impracticable for numerous reasons but it is possible temporarily to reduce capillary and venous pressure by applying suction to the extremities.

An aluminium box was built large enough to accommodate the lower extremity to a point about 6 inches above the knee and strong enough to withstand pressures of —120 to +120 mm. Hg. A mercury manometer, communicating with the interior of the box, was equipped with electrodes so arranged that the one-half horse power motor operating an air pump was stopped through an electrical relay, whenever the pressure in the box exceeded or fell below atmospheric pressure by 120 mm. Hg. A valve was inserted between the pump and the box so that for 25 seconds the pump evacuated air from the box while for 5 seconds the pump expelled cooled air into the box.

† The theelin and antuitrin "S" used in these experiments was generously supplied to us by Parke, Davis and Co., through the kindness of Dr. Oliver Kamm.

The effects of alternate suction and pressure were first studied by using a circulation schema in which a rubber bag simulated the distensible capillary and venous bed. The rate at which water flowed through the system under a pressure of 80 mm. Hg. was measured first without external pressure variations and then for a like period with alternate suction and pressure. During the latter period the total outflow was increased by between 60 and 80%, indicating the correctness of the working hypothesis.

In order to test the effects of alternate suction and pressure on local circulation the leg was inserted into the aluminium box through the opening of a rubber diaphragm and exposed alternately to suction (—100 to —120 mm. Hg. for 25 seconds) and to pressure (+80 to +120 mm. Hg. for 5 seconds), while skin temperature was measured to detect changes in blood flow. The short periods of positive pressure were employed in order to empty the engorged capillaries and veins of blood drawn in by the negative pressure so that the next period of suction might again fill the peripheral vascular bed with fresh arterial blood. Continuous suction could not be expected to increase total blood flow except during the brief period required to distend the veins and capillaries.

The left leg was placed in the aluminium box while the right leg was placed in a control box of similar shape and size. Compression and expansion of the air within the aluminium box caused air temperature to fluctuate but by means of electric lamps the control box was always kept slightly warmer than the aluminium box.

TABLE I.

Room temp. °C.	Digit	Skin temp. beginning of suction and pressure. °C.	Fall in temp. during suction and pressure. °C.	Av. temp. of air in box. °C.
12.2-13.0	L1 suction and	33.0	—1.3	19.2
	L3 pressure	32.3	—2.7	
	R1 control	33.9	—5.6	20.8
	R3	33.8	—7.4	
12.0-13.0	L1 suction and	32.7	—2.8	19.2
	L3 pressure	32.5	—6.4	
	R1 control	32.8	—7.4	20.8
	R3	32.5	—8.9	
11.3-13.1	L1 suction and	33.4	—1.7	20.2
	L3 pressure	32.7	—2.9	
	R1 control	34.8	—4.5	22.2
	R3	33.7	—6.4	

The effects of alternate suction and pressure on the rate of cooling of an extremity previously warm are shown in Table I. With the right, or control, leg in a slightly warmer environment the left leg was exposed to suction and pressure for 22 to 58 minutes while the extremities cooled. The right toes cooled rapidly (Table I) while the left toes cooled more slowly, remaining from 1.4 to 4.5°C. warmer. The temperature of the aluminium box was always lower than that of the control box. The air in the aluminium box was moving whereas that in the control box was still. The left extremity was slightly congested by the rubber cuff while the right was not. All of these factors would favor more rapid cooling of the left extremity but nevertheless the left extremity remained definitely warmer as long as suction and pressure were continued. In view of control experiments it is believed that this was due to increased blood flow produced by the alternating periods of suction and pressure.

The practical value of this method of increasing blood flow is to be tested in patients with obliterative structural disease of the arteries of the extremities. It is possible that improved circulation accompanied by repeated distention of those blood vessels which are not entirely rigid due to organic disease may favor the development of collateral circulation and may thus delay or prevent the advance of threatened gangrene.

6587

Incidental Hyperguanidinemia in Dogs in Parathyroid Tetany.

W. RAY BRYAN AND A. S. MINOT. (Introduced by W. E. Garrey.)

From the Departments of Physiology and Pediatrics, Vanderbilt University School of Medicine, Nashville, Tennessee.

In the course of studies of the chemical composition of the blood to determine the relative importance of certain variations which may contribute to the picture of tetany following parathyroidectomy the guanidine concentration has been determined. The interest in guanidine bases originated in the work of Paton and collaborators[1, 2] who believed that guanidine accumulated as a result of parathyroid deficiency and was the cause of parathyroid tetany.

[1] Paton, D. N., and Findlay, L., *Quart. J. Exp. Physiol.*, 1916, **10**, 318.
[2] Paton, N., *Edinburgh Med. J.*, 1924, **31**, 541.

TABLE I.
Blood Guanidine Determinations in Different Dogs before Parathyroidectomy and During Tetany.*

Blood Guanidine, mg. per 100 cc. Before Oper.	Tetany	Blood Guanidine, mg. per 100 cc. Before Oper.	Tetany
——	0.37	0.45	0.72
——	0.43	0.45	0.62
——	0.41	0.44	1.00
——	0.37	0.36	0.37
——	0.40	0.37	0.61
——	0.90	0.39	0.43
——	0.84	0.33	0.87
——	0.34	0.38	0.43
0.37	0.46	0.37	0.80
——	0.46	0.33	0.37
——	0.56	0.37	0.58
——	0.75	0.42	0.38
0.37	0.40	0.39	0.42
——	0.80	0.41	0.81

* Experiments performed between January, 1931, and March, 1932.

The method we used for the determination of guanidine was that of Major and Weber,[3] with minor adaptations as described by Minot and Dodd.[4] Our results on 28 parathyroidectomized dogs are shown in Table I. Although in some instances the guanidine was not determined in the blood before operation the figures which are presented on normal dogs are within the limits of 0.35 to 0.45 mg. per 100 cc., which we have found to be the normal range as determined by this method in a large number of dogs. The results in the column marked "tetany" were obtained on samples of blood drawn when the symptoms were of varying intensity. Some were taken at the onset of symptoms, some during mild tetany and others during severe general convulsions. About 50% of the dogs in this series show an increase in blood guanidine. The rest, although showing equally typical and severe symptoms of tetany, had no significant increase in guanidine. Hyperguanidinemia, therefore, cannot be the basic cause of parathyroid tetany.

The vast amount of work which has been reported in the literature indicates clearly that parathyroid tetany results primarily from a disturbance in calcium metabolism. Furthermore the only known function of the parathyroid glands is their rôle in regulating the calcium metabolism in the body. However, from what is known of the toxicology of guanidine[5,6] and the antagonism between the

[3] Major, R. H., and Weber, C. J., *Johns Hop. Hosp. Bull.*, 1927, **40**, 87.
[4] Minot, A. S., and Dodd, K., *Am. J. Dis. Child.*, in press.
[5] Frank, Stern, and Nothmann, M., *Z. f. d. ges. exp. Med.*, 1921, **24**, 341.
[6] Paton, N., *Glasgow Med. J.*, 1925, **104**, 297.

action of this toxic base and that of a calcium salts[7, 8] it is likely that guanidine when its concentration in the blood is appreciably increased tends to increase the severity and hasten the onset of tetany.

Experiments which demonstrate this relationship and show the conditions under which the incidental hyperguanidinemia occurs will be reported subsequently.

6588

The Hydrogen-ion Concentration and Buffer Capacity of Oyster Liquor of the Chesapeake Bay.

JOHN C. KRANTZ, JR. (Introduced by E. Uhlenhuth.)

From the Bureau of Chemistry, State of Maryland Department of Health.

The hydrogen-ion concentration of oyster liquor is determined in the routine examination of oysters as a measure of the stage of decomposition.[1] A range of hydrogen-ion concentration has been established for "Good Oysters" between pH 6.25 and 7.00 (cresol red indicator). Stale oyster liquor and slightly sour oyster liquor exhibit a higher hydrogen-ion concentration. KoKubo[2] studied the pH of the blood and pericardial fluid of the *Ostraea circumpicta* and found the former to be pH 7.24 and the latter pH 7.16.

The purpose of this investigation is to determine the pH and buffer capacity of the fresh oyster liquor of *Ostraea virginica* from the Chesapeake Bay and to study their variations in different natural environments.

Experimental. The oysters were collected during the winter months and preserved at a temperature not exceeding 5°C. The determinations were made at a period not exceeding 12 hours after collection. The sea water was taken from above the oyster beds at the time the oysters were collected.

The hydrogen-ion concentration determinations were made immediately after the liquor was taken from the shell. The determinations were carried out on the composite liquor of at least 12 oysters.

---

[7] Kühnau, J., and Nothmann, M., *Z. f. d. ges. Exp. Med.*, 1924-5, **44**, 505.

[8] Minot, A. S., and Cutler, J. T., *J. Clin. Invest.*, 1928, **6**, 369.

[1] Hunter, A. C., and Linden, B. A., *Am. Food J.*, 1923, **18**, 538.

[2] KoKubo, Seiji, *Science Repts., Tohoku Imp. Univ.*, 4th series, 1929, **4**, 207, through *Chem. Abs.*, 1929, **23**, 4274.

The hydrogen-ion concentration of the liquor and dilutions was determined by means of the following set up

$$Pt|(QH\ satd.),\ H^{+}(a_1)||KCl\ satd.\ Hg_2Cl_2|Hg$$

at 25° ± 0.5°C. A saturated potassium chloride agar bridge was employed. The Leeds and Northrup laboratory type potentiometer was used. The readings were reproducible to 0.02 pH.

The hydrogen-ion concentration of the sea water was determined colorimetrically by comparison with standard buffer solutions. No correction for salt-error was made. The values were reproducible within 0.1 pH.

In 1925 Ramage and Miller[3] determined the salt error of cresol red in solutions of varying degrees of salinity. The average concentration of NaCl in the Chesapeake area from which these oysters were taken is about 6 gm. per L. or approximately 0.1 M. NaCl. Likewise, the ionic strength of the solution would closely approximate 0.1. Ramage and Miller set the salt error for cresol red at this salinity at —0.12 pH. However, as pointed out by Kolthoff, (pH and Electrotitration p. 56) at this concentration the error is practically zero as the ionic strength of the buffers used in comparison are from about 0.08 to 0.1 ionic strength.

In this work as the buffer comparisons and the solutions meas-

TABLE I.

No.	Date	Area	pH of liquor	pH of sea water
1	1/ 9/31	Choptank River	6.95	8.2
2	1/29/31	Tangier Sound	6.95	8.3
3	2/10/31	Miles River	6.95	8.5
4	2/13/31	Severn River	6.95	8.5
5	3/ 6/31	Harris Creek	6.95	8.7
6	10/29/31	Choptank River	6.93	8.5
7	11/11/31	Tangier Sound	7.08	8.9
8	11/24/31	Magothy River	7.15	8.2
9	11/19/31	Back Creek	6.97	8.2
10	11/24/31	Bodkin Creek	6.92	8.2
11	11/27/31	Miller's Island	6.88	8.1
12	12/ 5/31	Chincoteague	6.92	8.2
13	12/15/31	Back Creek	6.95	8.2
14	1/13/32	Severn River	7.06	8.2
15	1/14/32	Rock Point	6.87	8.2
16	1/22/32	South River	6.94	8.2
17	2/ 8/32	Holland Point	6.98	8.0
18	2/ 9/32	Upper Steps	6.92	8.1
19	2/18/32	Choptank River	6.98	8.2

Median	6.95	P. E. of Mean	±0.011
Mode	6.95	P. E. of Standard Deviation	0.007
Mean	6.96	Coefficient of Variability	1.0
Standard Deviation	0.07		

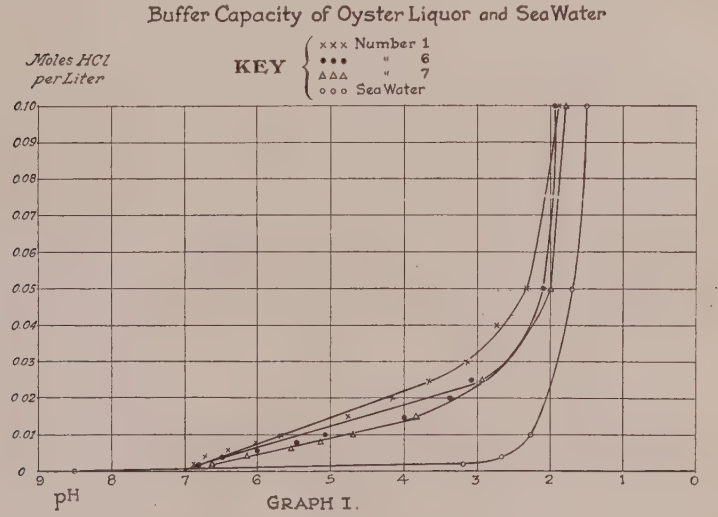
[3] Ramage and Miller, *J. Am. Chem. Soc.*, 1925, **47**, 1230.

ured were of approximately the same ionic strength pH = log [aH^+], corrections were unnecessary in the author's opinion. The slight variations between pH and —log [aH^+] were of the magnitude of ±0.02 pH.

Results. 1. *Hydrogen-ion concentration of oyster liquor.* (See Table I.)

2. *Buffer capacity of oyster liquor.* The buffer capacities of samples Nos. 1, 6 and 7 were determined by the addition of hydrochloric acid according to the method of Van Slyke.[4] With sample No. 6 the buffer capacity of the sea water was determined in this area as has been done by other investigators[5] in other areas.

The results are shown in Graph 1.



GRAPH 1.

From the curves in Graph 1 the average buffer capacity of these 3 samples of oyster liquor at approximately pH 7.0 is $\beta = -dB/-dpH = 6.3 \times 10^{-3}$. On the other hand the value of β for sea water is 3.6×10^{-4} at pH 8.5.

With 13 samples a long range average buffer capacity was determined by adding hydrochloric acid in the ratio of 0.01 mole to one liter of liquor. These values are shown in Table II.

Summary. The hydrogen-ion concentration of the liquor of *Ostraea virginica* taken from various portions of the Chesapeake

[4] Van Slyke, D. D., *J. Biol. Chem.*, 1922, **53**, 528.

[5] Thompson, T. G., and Bonnar, R. U., *J. Ind. Eng. Chem. Analyt.*, 1931, **3**, 393.

TABLE II.

No.*	pH of liquor	pH after addition	$\frac{-\Delta B}{-\Delta pH} \times 10^{-3}$
4	6.95	5.64	7.6
8	7.15	4.85	4.3
9	6.97	4.55	4.1
10	6.92	5.30	6.2
11	6.88	4.70	4.6
12	6.92	3.50	2.9
13	6.95	3.15	2.6
14	7.06	4.30	3.6
15	6.87	4.05	3.5
16	6.94	4.85	4.8
17	6.98	4.10	3.5
18	6.92	2.75	2.4
19	6.98	3.50	2.9
			Mean 4.1×10^{-3}

* Numbers are taken from Table I.

Bay shows little variation from the established norm of pH 6.95. The hydrogen-ion concentration of the liquor was not influenced by the hydrogen-ion concentration of the environmental sea water in the number of samples studied. The buffer capacities of the oyster liquors showed a wide variation.

The author wishes to acknowledge his indebtedness to Mr. S. J. Caskey for the collection of the oysters, and to Mr. J. F. Muller for the preparation of the graph, each from the Bureau of Sanitary Engineering of the State of Maryland Department of Health.

6589

Carbohydrate Fractions from Vibrio Cholerae, and Related Organisms.*

RICHARD W. LINTON AND D. L. SHRIVASTAVA.

From the All-India Institute of Hygiene and Public Health, Calcutta.

We have shown[1] that the carbohydrate substances extracted from *Vibrio cholerae*, and related organisms, are so closely allied that cross-reactions will occur between them and immune sera at high dilutions. This cross-relationship was independent of the agglutination reaction, to differentiate the presumably pathogenic from

* This work was done under the auspices and with the support of the Indian Research Fund Association, and is published by permission of the Secretary.

[1] Linton, R. W., *Ind. J. Med. Res.*, 1932, **20**, 347.

non-pathogenic vibrios, since carbohydrate from a water vibrio, for example, would precipitate with serum against a pathogenic form, or against a rough non-agglutinable form. The vibrios included pathogenic smooth agglutinating forms, rough forms, some of which agglutinated and some of which did not, from human sources and from water, and non-agglutinating smooth water vibrios. These vibrios were characterized by the possession of a carbohydrate which was very similar if not identical in all.

A closer study has now been made of the carbohydrates of these organisms. The method of carbohydrate extraction will be given in detail subsequently.[2] The 48-hour growth of 300 to 500 Roux flasks is washed off in normal saline, of which 10 cc. or less is used for each bottle. Glacial acetic acid is added to the washings to make a N/20 solution, and the organisms are then boiled under reflux until coagulation. Upon cooling, clumps fall to the bottom and leave a clear yellow supernatant fluid. Coagulation time varies with the type of organism. With rough organisms coagulation and clumping may take place between 10 minutes and one hour; smooth organisms, from 3 to 6 hours. The organisms are removed from the extract in a Sharples supercentrifuge, and the extract filtered to a water clearness in Seitz filters. After concentration to approximately 500 cc., the extract is precipitated with 3 volumes of absolute alcohol. The alcohol precipitation is repeated at least 6 times and the usual protein reactions become negative after the second or third precipitations. The water solution, each time after the first, is concentrated to 250 cc. The final precipitate is dried *in vacuo*, and yields in some cases a brittle brownish gum, translucent in thin layers, and in other cases a white friable powder. The former is found in extracts from the non-pathogenic vibrios, and the latter appears in extracts of the pathogenic forms. The yield is between 10 and 15 gm.

After hydrolysis with normal sulphuric acid for 2 or 3 hours on sandbath, the yield of reducing substances is usually between 30% and 40%, calculated as glucose. By fractional precipitation with absolute ethyl alcohol, the hydrolysate may be separated into 3 portions. Fraction I, which separates from the hydrolysate upon the addition of 3 volumes of alcohol, is a dark reddish gum, containing no reducing substances even after prolonged boiling with acid. Fraction II, which separates upon the addition of 6 volumes of alcohol, and Fraction III, which remains behind in the hydrolysate,

[2] Linton, R. W., and Shrivastava, D. L., *Ind. J. Med. Res.*, in press.

have been qualitatively determined, and the results are given in Table I, for 4 different vibrios. Fraction II is the same in all the organisms, and appears to consist of glycuronic acid and galactose. It appears to be an aldobionic acid of the type found widely among bacterial carbohydrates and plant gums. The reducing power of this fraction is increased by 25% to 30% by prolonged boiling or autoclaving in acid solution, indicating that the compound is undergoing further hydrolysis. It has also been separated from a mixture of Fractions II and III as the barium salt.

TABLE I.

Analysis of Fractions II and III of *Vibrio cholerae*, and Related Organisms.

	Fraction II	Fraction III
1. A smooth agglutinating vibrio, recently derived from a case of cholera.	glycuronic acid + galactose	galactose
2. A rough, non-agglutinating strain derived from the above by the action of bacteriophage.	,, ,, ,, ,,	,,
3. A rough, weakly agglutinating strain.	,, ,, ,, ,,	arabinose
4. A water vibrio, smooth and non-agglutinating.	,, ,, ,, ,,	,,

The third fraction of the hydrolysate consists of a single sugar, which differs according to the type of vibrio from which the carbohydrate has been derived. In a smooth, agglutinating vibrio recently derived from a case of cholera, this fraction consisted of galactose. The same sugar is found in a rough non-agglutinating vibrio which is a secondary growth obtained from the first after the action of bacteriophage. The third organism in the table is a rough, weakly agglutinating strain, whose complete history cannot be traced, but which is supposed to be non-pathogenic. In it Fraction III consisted of arabinose. The last organism is a smooth, non-agglutinating water vibrio, in which Fraction III also contained arabinose.

Complete details as to the identification of these substances will be given later.[2] The following compounds have been prepared, and their melting points determined. For galactose, phenylosazone, methylphenylhydrozone, *o*-tolylhydrozone, mucic acid, and the formation of crystalline galactose; for arabinose, the phenylosazone, the diphenylhydrozone, and the formation of the crystalline sugar itself; for glycuronic acid, potassium acid saccharate, the barium *p*-bromphenylhydrozone of glycuronic acid, and the phenylosazone,

which also gave the characteristic reaction with naphthoresorcin and benzol.[3]

While the vibrios contain carbohydrate factors so closely allied that they cross-react throughout the group at high dilution, yet the vibrios themselves are not identical, since their specific substances are different. So far as our present analysis goes, the chemical difference between the specific substances of the pathogenic vibrio and the water vibrio lies in the possession of galactose by the first and of arabinose by the second. It appears further that the smooth-rough transition, brought about by bacteriophage action, does not change the character of the carbohydrate, nor does the change from the agglutinable to the inagglutinable type have any effect upon the specific substance.

6590

Studies on Sensitization. I. Skin Sensitivity and Serum Precipitin Response Following Intracutaneous Injections.*

R. L. KAHN.

From the Department of Bacteriology, University of Michigan.

This report is the first of a series undertaken with the aim of throwing light on the rôle of sensitization in infection and immunity. Our early studies deal with the tissue hypersensitiveness that results from repeated injections of protein substances in animals, a condition generally classed under the Arthus phenomenon. Albino rabbits served as the experimental animals and the sensitizing injections were made, in most instances, intracutaneously.[1] The white skin in these animals rendered the effects of the injections readily discernible. This fact, in turn, permitted quantitative studies of the skin (local) response and its ready correlation with the serum (general) response.

The present study utilizes the method for measuring skin sensitivity recently reported.[2] Briefly, into the hair-clipped skin of an

[3] Abderhalden, E., *Handbuch d. biol. Arbeitsmethoden,* Abt. I, Tl. 5, Kohlenhydrate, 99.

* This series of studies was aided by grants from the Commonwealth Fund of New York and the Faculty Research Fund, University of Michigan.

[1] A review of the literature on immunization by cutaneous means is given by Tuft, L., *J. Immunol.,* 1931, **21**, 85.

[2] Kahn, R. L., *J. Bact.,* 1933, **25**, 81.

albino rabbit are injected 0.1 cc. quantities of a series of dilutions of some protein with physiological salt solution. The dilutions usually employed are: undiluted, 1:10, 1:100, 1:1,000, 1:10,000, and 1:100,000. The skin response is read 24 hours after the intracutaneous injections, and the highest dilution producing an inflammatory area, is termed the skin sensitivity titer of the rabbit.

If, for example, human serum previously heated for 30 minutes at 56°C. is used as the protein, the skin of a normal rabbit will show practically no response to this sensitivity test; only the area wherein the undiluted serum had been injected will show a slight transitory edema. A rabbit previously sensitized to human serum, however, will be likely to show, after a similar sensitivity test, a marked inflammatory response with central necrosis in the area where the 0.1 cc. of undiluted serum had been injected; a somewhat less marked response in the area where the 0.1 cc. of the 1:10 serum dilution had been injected, with gradual reduction in the response corresponding to the higher dilutions of serum, showing perhaps only a small erythematous area in the 1:10,000 dilution, with no response in the 1:100,000 dilution. A rabbit showing this picture is recorded as having a skin sensitivity titer of 10,000 to human serum.

The diameters of the inflammatory areas, resulting from skin sensitivity tests with human serum, in 4 rabbits having sensitivity titers of 10,000, 10,000, 100,000, and 100, respectively, are illustrated in Table I. It should be emphasized that while necrosis accompanies inflammation in the low dilutions of serum, the responses in the higher dilutions are limited to inflammation.

TABLE I.
Skin Reactions in Rabbits Sensitized to Human Serum.

Rabbit	Serum Dilutions						
	Und.	1:10	1:100	1:1,000	1:10,000	1:100,000	1:1,000,000
	Diameter of inflammatory area expressed in cm., 24 hours after intracutaneous injections of 0.1 cc. doses of serum dilutions.						
1	5.5	5	3	2	1	—	—
2	3	3	2.5	1.5	1	—	—
3	4	2	1.5	1.5	1.5	1.5	—
4	2.5	1.5	1	—	—	—	—

It is not aimed to imply that the skin sensitivity titer is an absolute criterion of the degree of sensitization in rabbits. It is believed, however, on the basis of our present knowledge, that a rabbit showing a skin response to a 1:1,000,000 dilution of a protein is probably sensitized to a greater degree, for example, than a rabbit showing a skin response to a 1:1000 dilution.

Intracutaneous Injections of Serum and Egg White. An intracutaneous injection of these protein solutions in amounts from 0.5 cc. to 2 cc. in rabbits, will produce after 6 to 14 days a precipitin titer ranging from 1,000 to 100,000 and a skin sensitivity titer from 10 to 1,000. A repeated sensitizing injection of the protein, in about 2 weeks will frequently raise the skin sensitivity titer to about 100,000 with an equally high or higher serum precipitin response. After a week or more, the latter response is likely to be considerably reduced while the skin sensitivity response usually remains at a high level for a considerable period.

Intracutaneous Injections of Gelatin and Split Protein Products. Gelatin, as is well known, lacks cystine, tyrosin and tryptophane, 3 important aminoacids. This incomplete protein, according to Wells,[3] does not produce anaphylactic antibodies in guinea pigs. Neither, apparently, does it produce complement fixing substances in rabbits.[4] It is generally accepted also that split protein products do not produce antibodies. Intracutaneous injections of 1 cc. and 0.5 cc., in series, 14 days apart, of a 7.5% solution of gelatin of high purity, given in fractional doses of 0.2 cc., did not produce serum precipitins but the skin gave a mild sensitivity response. Before the sensitizing injections, the rabbit's skin gave no response to 0.1 cc. containing 7.5 mg. of gelatin. Two weeks after the second injection, the rabbit showed a skin sensitivity titer of 10, corresponding to 0.75 mg. of gelatin. Racemized casein[5] gave no skin sensitivity (nor precipitin) response after 2 intracutaneous injections, 17 days apart, of 3 cc. quantities, each containing 60 mg. Negative results were also obtained following intracutaneous injections of similar quantities of: (1) proteose formed during preparation of racemized casein; (2) proteose formed from gliadin by acid digestion; (3) proteose formed in preparation of racemized zein; (4) proteose soluble in alcohol, obtained during racemization of edestin† It is possible that larger amounts of these products and continued injections might bring forth a skin sensitivity response. It should be mentioned, however, that 2 injections of 20 mg. each of casein (Kahlbaum), 17 days apart, gave, 2 weeks after the second injection, a skin response of 100, corresponding to 0.007 mg. of this protein. Previous to the sensitizing injections, the same rabbit gave

[3] Wells, H. G., *J. Infect. Dis.*, 1908, **5**, 449.

[4] Kahn, R. L., *J. Immunol.*, 1918, **3**, 277.

[5] Ten Broeck, C., *J. Biol. Chem.*, 1914, **17**, 369.

† These split proteins were kindly furnished by the late Dr. Frank P. Underhill.

no skin response to 0.7 mg. of the same protein. The question of the sensitizing capacity of gelatin and split proteins merits further investigation.

Intracutaneous Compared with Intravenous Injections. No essential differences in skin sensitivity and in serum precipitin response were noted following these 2 methods of sensitization (Table II).

TABLE II.
Serum Precipitin and Skin Sensitivity Titers

Rabbit	Method of Injection	Dilutions human serum 1 cc. amounts injected 14 days apart	Response days after 2nd injection 0 days serum p'ptin	14 days skin s'tivity	14 days serum p'ptin
95	Intracut.‡	1:10	100,000	100	1,000,000
161	Intraven.	1:10	10,000	100	1,000,000

‡ The 1 cc. amount was given in fractional doses of 0.2 cc.

This table also shows that high precipitin titers can be obtained with small sensitizing doses of the proteins, contrary to a widely held belief that it is necessary to give multiple injections of comparatively large amounts to produce high titers. It should be emphasized, however, that variation in skin sensitivity and precipitin titers occurs in rabbits even when the sensitizing injections are made under identical conditions.

6591

Studies on Sensitization. II. Skin Sensitivity and Serum Precipitin Response Following Injections of Unheated and Heated Serum.

R. L. KAHN.

From the Department of Bacteriology, University of Michigan.

Fresh, unheated human serum is toxic to the rabbit's skin. If 0.1 cc. of such serum is injected intracutaneously, a marked inflammatory response, boil-like in appearance, measuring from 1 to 2 cm. in diameter, follows with subsidence in about 10 days or longer. When human serum is previously heated for 30 minutes at 56° C., however, it has practically no toxic effect on the rabbit's skin. Intracutaneous injection of 0.1 cc. produces a slight transitory thickening of the skin which usually disappears in 24 hours. The question arose, whether the inflammation accompanying the injection of

unheated serum might serve as a stimulus to the skin sensitivity and serum precipitin responses of the rabbit. Accordingly, one group of 8 rabbits was injected with unheated serum and a corresponding group with heated serum. Usually 2 injections were employed, the first one being 1 cc., and the second (10 or 14 days after the first), 0.5 cc. These amounts were given intracutaneously in fractional doses of 0.2 cc. The results as demonstrated in the accompanying table, show but little difference in the skin sensitivity

TABLE I.

Skin Sensitivity and Serum Precipitin Response Following Intracutaneous Injections of Unheated and Heated Serum

Rabbit	Serum	1st injection, 1 cc.; 2nd inj., 10 days later, 0.5 cc.	Skin reactions following intracutaneously given fractional doses.	Response—days after 1st injection					
				10		30		45	
				Skin s'tivity	Serum p'pitin	Skin s'tivity	Serum p'pitin	Skin s'tivity	Serum p'pitin
33	Unheated	1st inj.	Boil-like areas 1.5 cm. diam. necrotic centers, subsidence in 10 days						
		2nd "	Large confluent mass, 10 cm. diam. necrotic centers at points of injection, subsidence in 20 days	100	10,000	10,000	100,000	10,000	10,000
35	Heated 30 min. 56°C.	1st "	Slightly thickened pink areas, subsidence in 24 hours						
		2nd "	Raised inflamed areas 1.5 cm. diam., subsidence in 5 days	10	100,000	100	10,000	1,000	10,000

and serum precipitin titers following the injection of these 2 types of serum.

6592

Studies on Sensitization. III. Skin Sensitivity in the Absence of Serum Precipitins.

R. L. KAHN.

From the Department of Bacteriology, University of Michigan.

It is generally assumed that the tissue hypersensitiveness which follows repeated injections in rabbits of substances protein in nature, is accompanied by the presence of serum precipitins in these animals. Opie[1] claims that the local inflammatory response in sensitized animals is due to the meeting of the serum precipitin with the antigen in the tissues. In our studies, we have constantly found serum precipitins to accompany skin sensitivity in the early weeks of sensitization. But with time, especially after a lapse of several months following the initial sensitization of the rabbits, skin sensitivity is frequently present in the total absence of serum precipitins. The accompanying table gives an illustration of such findings.

TABLE I.
Skin Sensitivity and Serum Precipitins Following a Prolonged Period after Discontinuance of Sensitizing Injections.

Rabbit	Weeks after Sensitizing Injections	Skin Sensitivity	Serum Precipitin
176	14	10,000	—
198	11	1,000	—
209	8	10,000	und.*
211	8	100,000	—
212	8	100,000	—
215	8	10	—

* Precipitation obtained with undiluted serum only.

Studies now in progress indicate that sensitization following injection of protein, frequently precedes by a few days the appearance of serum precipitins. Soon, the latter reach a high level—even up to 1:1,000,000 dilution, or higher—the skin sensitivity titer usually lagging. Shortly the serum precipitin titer begins to drop, gradually becoming negative. The skin sensitivity titer, however, remains at a fairly high level for many months. No data are available at pres-

[1] Opie, E. L., *J. Immunol.*, 1929, **17**, 329.

ent as to the length of time a rabbit will remain sensitized after the protein injections have been discontinued.

6593

Studies on Sensitization. IV. Antitoxin Immunity Contrasted with Tissue Hypersensitiveness.

R. L. KAHN.

From the Department of Bacteriology, University of Michigan.

In connection with our studies of tissue hypersensitiveness following repeated injections of protein in rabbits, it seemed of interest to obtain a picture of antitoxin immunity in the same animal that is sensitized to some protein. A quantitative method for measuring skin immunity to diphtheria toxin, based on the Schick test, and for measuring the amount of antitoxin present in the serum of the immune animal, following intracutaneous injections of toxoid has been described.[1] Briefly, the least quantity of diphtheria toxin, in a 0.1 cc. volume, that is capable of producing an erythematous response following an intracutaneous injection in a normal rabbit is determined. This quantity usually ranges from 0.000003 MLD to 0.0000003 MLD. A given amount, such as 0.5 cc. of toxoid is then injected intracutaneously in fractional doses of 0.25 cc. in the same rabbit. After about 3 weeks, it will be found that the least quantity of diphtheria toxin that will produce erythema following intracutaneous injections of 0.1 cc. amounts, is considerably higher than 0.000003 MLD. With an additional injection of toxoid, the amount of toxin necessary to produce an erythematous response might be 0.1 or 1 MLD. With continued injections of toxoid, a skin response may not be obtained until 5 or 10 MLD of toxin are injected intracutaneously.

Parallel with this picture of the development of skin immunity to the toxin is the increase of antitoxin in the blood serum of the immunized animal, also measured by intracutaneous means, and determined as follows: A small dose of diphtheria toxin is chosen that invariably produces a definite inflammatory response in the skin of a normal rabbit when given intracutaneously in a 0.1 cc. amount. This dose, 0.0003 MLD, is mixed in series with varying dilutions

[1] Kahn, R. L., *J. Bact.*, 1933, **25**, 81.

of immune rabbit serum. Thus, 0.1 cc. of the toxin dilution containing 0.0003 MLD is mixed with 0.1 cc. of undiluted immune rabbit serum. Similarly, 0.1 cc. of the same toxin dilution is mixed with 0.1 cc. of 1:10 dilution of the immune serum. A third mixture is prepared with 1:100 dilution of the immune serum. Similar mixtures with still higher dilutions of the immune serum are prepared when indicated. The 0.2 cc. amounts containing the toxin and immune serum are injected intracutaneously in a normal rabbit with proper controls. The highest dilution of the immune serum capable of neutralizing the dose of toxin gives a measure of the amount of antitoxin in the immune serum. By utilizing this method, it will be found that whereas the rabbit before the toxoid injections showed no antitoxin in its serum, after the toxoid injections, sufficient antitoxin may be present in the serum to neutralize the 0.0003 MLD of toxin in a serum dilution as high as 1:10,000 or more.

Table I gives a record of a rabbit which, following a series of intracutaneous injections of toxoid* and, at a later date, intravenous injections of serum (previously heated at 56°C. for 30 min.), shows the presence of a highly developed skin immunity to diphtheria toxin as well as a high toxin neutralizing capacity of the

TABLE I.

Immunization and Sensitization of Rabbit (108) Following Injections of Toxoid and of Human Serum.

Date 1932	Intracut. injections of toxoid; fractional doses 0.25 cc. Injection, cc.		Intraven. injections of serum Injection cc.		Immunization response to toxin		Sensitization response to serum	
					Smallest amt. diphtheria toxin producing erythema MLD.	Highest dilution rabbit serum neutralizing 0.0003 MLD. toxin	Titer of skin sensitivity	Precipitin titer rabbit serum
6/16	1st	1						
6/20					0.0000003	no neutralization		
7/ 6					0.001	undiluted		
7/11	2nd	0.5						
8/ 2					1.0	1:100		
8/15	3rd	0.5						
10/ 4					2.5	1:1,000		
10/14								Negative
10/16			1st	1				
10/22	4th	0.5						
10/25					2.5	1:1,000	1:100	1:100,000
10/26			2nd	0.5				
11/ 9					7.5		1:100	1:100,000
11/22	5th	0.5						
12/ 1			3rd	1				
12/12					7.5	1:10,000	1:100	1:100,000

* The diphtheria toxin, toxoid and antitoxin were kindly furnished by the Bureau of Laboratories, Michigan Department of Health, Lansing.

serum. At the same time it shows also a moderate skin sensitivity titer and a high precipitin titer. No skin sensitivity test was given this rabbit before administering the sensitizing doses of serum, because this experiment was part of a group where sensitization was attempted purely by the intravenous route.

Employing this method for demonstrating the immunity of the skin to diphtheria toxin and the capacity of the serum to neutralize this toxin, combined with tissue hypersensitiveness and precipitin production, it appears that the immunity has little effect on the sensitivity, and vice versa.

6594

Studies on Sensitization. V. Fixation of Diphtheria Antitoxin in Skin of Rabbits Sensitized to Horse Serum.

R. L. KAHN.

From the Department of Bacteriology, University of Michigan.

The element of fixation that accompanies sensitization was well demonstrated by Opie,[1] who cut out the inflammatory area resulting from subcutaneous injection of the specific protein in a sensitized rabbit, extracted the ground tissue with salt solution, and established the presence of the antigenic substance in the fluid by testing it with the serum of a specifically sensitized rabbit. In this laboratory, the aspect of fixation was studied from another point of view. The question was raised, whether an animal sensitized to horse serum would fix diphtheria antitoxin at the point of injection. Accordingly, the following conditions were experimentally established: (1) 50 MLD of diphtheria toxin injected intracutaneously in rabbits of about 3 kg. weight, cause death in from 2 to 4 days. (2) The same dose of toxin and 50 units antitoxin injected intracutaneously 1 inch apart, result in the survival of the animals.

A group of rabbits previously sensitized to horse serum was given the toxin and antitoxin injections intracutaneously, employing non-sensitized rabbits as controls. It was found that the control animals survived this treatment while the sensitized animals, showing a marked inflammatory response at the area wherein the antitoxin was injected, succumbed within 2 to 4 days, unquestionably

[1] Opie, E. L., *J. Exp. Med.*, 1924, **39**, 659.

because the fixation of the antitoxin at the point of injection prevented its neutralizing action on the toxin. Table I gives the results obtained with 11 rabbits.

TABLE I.
Fixation of Diphtheria Antitoxin in the Skin of Rabbits Sensitized to Horse Serum.

Rabbit	Method of Sensitization cc. serum	Interval bet. last sensitizing injection and administration of toxin and antitoxin days	50 mld toxin and 50 units antitoxin given intracutaneously 3 cm. apart. Response to antitoxin injections	Condition of rabbit
77	1, horse intracutaneously	15	Marked inflammatory area 2x5 cm. diam.	Died on 4th day
77[1]	Non-sensitized control		Slight erythematous area 1 cm. diam.; negative after 24 hours	Survived
78	1 and 0.5, horse intracutaneously 3 weeks apart	2	Marked inflammatory area 3 cm. diam.	Died in 24 hours
78[1]	Non-sensitized control		Slightly thickened area 1.5 cm. diam., negative after 24 hours	Survived
80	1 and 0.5, horse intracutaneously 3 weeks apart	5	Marked erythematous area 3 cm. diam. with 1 cm. diam. blackened center	Died on 2nd day
80[1]	Non-sensitized control		Thickened area 2 cm. diam.; subsidence after 24 hours	Survived
117	1, human intracutaneously	16	Slightly thickened area, 1 cm. diam.; subsidence in 24 hours	"
118	" "	16	Slightly thickened area, 1 cm. diam.; subsidence in 24 hours	"
121	1, horse intravenously	14	Marked erythematous area 4 cm. diam.	Died on 2nd day
123	" "	14	Marked erythematous area 2.5 cm. diam.	Died on 4th day
125	" "	14	Mild response, 2 cm. diam. thickening of skin	Died on 4th day

Three rabbits sensitized intracutaneously with horse serum and 3 others sensitized intravenously, succumbed to the toxin and antitoxin injections. Three normal rabbits and 2 others sensitized to human serum survived the same treatment. Four of the rabbits sensitized with horse serum received but one injection of this reagent. One additional rabbit (79) sensitized to horse serum and one non-sensitized control (79[1]) received 50 units of antitoxin intracutaneously 48 hours before the injections of 50 units of antitoxin and 50 MLD of toxin. The sensitized animal died in 2 days, whereas the non-sensitized one survived. Autopsies were made of

the animals that had succumbed, with special attention to the gross appearance of the adrenals, which were found to be enlarged and hemorrhagic.

This study corroborates the generally accepted observation that the antitoxin molecule is bound to the protein (globulin) constituents of the immune serum. The study throws light also on conditions one frequently meets in children exposed to diphtheria, who after receiving diphtheria antitoxin for passive immunization, contract this disease some weeks later and are then given antitoxin intramuscularly. A severe local inflammatory response usually follows. The results of our experiments indicate that the children, when highly sensitized to the horse serum, do not derive any curative benefit from the antitoxin since this reagent undoubtedly remains fixed at the point of injection.

6595

Studies on Sensitization. VI. Specific Desensitization of Rabbits Sensitized to Protein Mixtures.

R. L. KAHN.

From the Department of Bacteriology, University of Michigan.

It is generally believed that while it is possible to desensitize rabbits injected with a purified protein such as crystalline egg albumin, it is not possible to desensitize these animals when injected with a complex mixture of proteins such as serum or egg white. On reinvestigating this problem, it was found that rabbits can be readily desensitized to serum and egg white, the desensitization, however, being of short duration. In the early experiments, large quantities, such as 10 or 20 cc. of these proteins were injected intravenously to produce desensitization. Later experiments indicated that relatively small amounts, such as 1 cc. or 0.5 cc. per kg. of body weight of rabbits, were sufficient to desensitize.

Table I illustrates desensitization in 2 rabbits, sensitized to human serum that had previously been heated for 30 min. at 56°C. Rabbit 173 had a skin sensitivity titer of 1,000, but no serum precipitins. By injecting intravenously 0.5 cc. of human serum per kg. of body weight, the skin sensitivity tests given 1 hour and 6 hours later, were practically negative, the skin response being limited to the injections of undiluted serum—a response commonly given by

TABLE I.
Desensitization of Rabbits by Intravenous Injections of Human Serum.

	Skin s'tivity	Serum p'pitin	Serum p'pitinogen
Rabbit 173 injected 0.5 cc. per kg; total 1.44 cc.			
Before injection	1,000	—	—
1 hr. after injection	und.*	und.*	100
6 " " "	und.	und.	100
24 " " "		10	10
48 " " "	100	10	10
120 " " "	1,000	10,000	und.*
Rabbit 213 injected 0.5 cc. per kg.; total 1.41 cc.			
Before injection	1,000	10,000	—
1 hr. after injection	—	—	100
6 " " "	und.	—	100
24 " " "		100	100
48 " " "	100	1,000	10
120 " " "	1,000	10,000	und.

* Reactions obtained with undiluted serum only.

normal rabbits. Forty-eight hours after the desensitizing injection, the skin response was 100; in 120 hours, it reached the original level. Of interest is the fact that the precipitin titer which was negative at the time of the desensitizing injection, reached 10,000 120 hours after this injection.

Rabbit 213 which showed a skin sensitivity titer of 1,000 and a serum precipitin titer of 10,000, was completely desensitized after an intravenous administration of 0.5 cc. human serum per kg. of body weight. One noted a return to the sensitized state 48 hours after the desensitizing injection.

It is evident also that the precipitinogen content of the serum is at its height during the desensitized state, disappearing as the titers of skin sensitivity and serum precipitins reach a high level.

6596

Dizziness, Fainting and Convulsions Due to Hyperactivity of the Carotid Sinus Reflex.

SOMA WEISS AND JAMES P. BAKER.

From the Thorndike Memorial Laboratory, Second and Fourth Medical Services (Harvard), Boston City Hospital, and the Department of Medicine, Harvard Medical School, Boston, Massachusetts.

A study has been made of the circulatory and the nervous systems of 12 subjects who complained of dizziness and fainting and in whom pressures of graded intensity on the right or left carot-

id sinus promptly and regularly induced asystole, fall in blood pressure (9 cases), dizziness (10 cases), fainting (8 cases), unconsciousness (7 cases), and convulsive movements (8 cases). The unconsciousness was preceded by typical and characteristic visual or auditory aurae. The convulsive movements were entirely contralateral on mild stimulation of the sinus, while on stronger stimulation they became generalized. After the pressure was relieved they ceased almost immediately. Dizziness and fainting were induced spontaneously by sudden turning of the head in 2 cases. The ages of the patients varied from 14 to 71 years; 10 were above 55 years.

A definite aneurysmal dilatation of one or both carotid sinuses was noted in 5 cases. A small tumor pressing on the sinus was found in 3 cases. No gross pathology of the sinus was detected in the remaining 4. Aneurysmal dilatation does not necessarily cause hyperirritability of the sinus reflex, for this lesion was observed in cases which failed to show hyperactivity of the sinus. Hyperactivity of the carotid sinus reflex is not always permanent, as was demonstrated by 2 cases which exhibited periodic hyperactivity of the reflex. The observations so far indicate that the hyperactivity of the reflex depends on the following factors, singly or combined: the excitability of the afferent nerve endings within the sinus, the state of the medullary center, and the excitability of the efferent cardiovascular nerve endings.

In some cases the convulsions depended on the duration of the asystole or the degree in fall of the arterial blood pressure. An asystole of 8 to 10 seconds was usually followed by convulsions. In one case fainting was observed without slowing of the heart or fall of the arterial blood pressure.

Comparative measurements of the volume and velocity of the blood flow indicate that the circulation time is considerably prolonged, and the cardiac output diminished, during the convulsive state. Internal jugular blood, taken during a convulsion, showed a decidedly lower oxygen and higher carbon dioxide content than when the patient felt well.

Instantaneous changes following pressure and release of pressure on the carotid sinus were observed in the cardiac rhythm and intracardiac conductive system. These changes in different cases consisted in the development and cessation of complete or partial A-V block, ventricular escape, complete temporary arrest of all chambers of the heart, bundle-branch block, and alterations characteristic of coronary artery disease.

In one case in which pressure of a small tumor on the sinus was

responsible for fainting and convulsions, surgical removal of the tumor and unilateral denervation of the sinus by Dr. Tracy J. Putnam abolished all sinus responses. Following section of the carotid nerve, this patient developed an acute arterial hypertension and auricular fibrillation; the hypertension disappeared within 48 hours, the fibrillation after 7 days. In this case complete relief followed the surgical treatment.

All the abnormal responses of the carotid sinus were abolished after unilateral novocainization of the sinus; while the reactivity of the opposite sinus remained unaltered or was increased. Intracarotid administration of a minute amount of atropine abolished ipsolaterally the effect of stimulation. Peripheral paralysis of the vagus endings by atropine abolished the cardiac effect but not the vasomotor. Epinephrine given parenterally abolished both the vasomotor effect and the cardiac slowing, presumably by overcoming the vagal inhibition.

Continuous intravenous administration of large amounts of histamine, acetylcholine, and carbon dioxide, which are dilators of the cerebral and peripheral blood vessels, failed to influence the effect of stimulation of the sinus.

The hyperactive state of the carotid sinus reflex is specific and is usually independent of the condition of other reflexes. In the cases studied, the somatic and other autonomic reflexes were normal. Conversely, in control subjects with sensitive and irritable vasomotor systems, the carotid sinus reflex was normal. In 150 cases of idiopathic epilepsy pressure on the carotid sinus failed to induce seizures.[1] Convulsions may be due to many causes, and hyperactivity of the carotid sinus reflex is seldom found when convulsions occur, but it can produce them.

[1] Lennox, W. G., personal communication.

6597

Argentaffin Cells of the Pancreas.

ERNEST VAN CAMPENHOUT. (Introduced by H. S. Burr.)
From the University of Montreal, Montreal, Canada.

In the pancreas of the dog, Lasowsky[1] has described the existence of argentaffin cells in the acini and in the islands of Langerhans. These cells do not reduce the silver solutions in the same way as the true argentaffin cells of the digestive tract; they remain unstained when placed in the ammoniacal silver solution but show up clearly after block silver treatment.

During an investigation of the histogenesis of the pancreas, we have been able to verify the descriptions of Lasowsky and to extend the facts to the human pancreas and to the pancreas of the calf, the pig and the chick; the Roger's silver impregnation, in addition to its nerve demonstration usefulness, brings the argentaffin cells into clear evidence.

The pancreas of a 12 mm. pig embryo shows numerous silver-stained cells; they represent the first histological differentiation in the solid epithelial cords, which later become the primary pancreatic ducts from which the islands and the acini will differentiate. The argentaffin cells can be found at all stages of development of pig and calf embryos and exist in the acini, the islets and the walls of the pancreatic ducts.

In the acini, the argentaffin cells are found between the secretory cells; they usually are pyramidal in shape, with their base extending along the basal part of the neighboring cells. The basal part of the cytoplasm is filled with granules, while these seem to be less closely grouped in the apical pole. Sometimes these cells have a multipolar aspect and send out granular processes which can be followed for a considerable distance (20 micra).

In the islands, the argentaffin cells show a smaller number of granulations which are scattered in the cytoplasm; the silver staining property appears to be uneven and one might consider the existence of intermediate stages between the clear and the argentaffin cells.

In the 10-day chick embryo, silver stained cells are exclusively found in the juxtahepatic region, where the first differentiation of the insular tissue takes place. In that area, they are numerous and show the same characters as the argentaffin cells in pig and calf embryos.

[1] Lasowsky, J. M., *Frankf. Z. f. Pathol.*, 1931, **140**.

In the adult human pancreas, the argentaffin cells are very numerous in the islands of Langerhans; few are found in the acini. In the regions where an acinoinsular transformation seems to take place, the intermediate elements are silver stained.

The distinction of clear and silver stained cells in the islets corresponds, probably, to the existence of alpha and beta cells, as shown by other techniques; the argentaffin properties will give an opportunity to study the cytological responses of the insular cells to various physiological stimuli.

The existence of the cells of Lasowsky in the acini is very difficult to ascertain without use of the silver reaction. The rôle of these acinian silver stained cells is unknown; in the light of our actual data we consider them as potential insular cells, which remain located in the acini, and which, under certain conditions, might be the source of fresh insular material.

6598

Successful Artificial Immunization of Dogs Against Taenia Echinococcus.

E. L. TURNER, D. A. BERBERIAN AND E. W. DENNIS.
(Introduced by W. S. Ladd.)

From the Department of Medicine, American University of Beirut.

The recent success of Miller[1] in immunizing rats against infestation with *Cysticercus fasciolaris* led us to consider the possibility of interrupting the life cycle of *Taenia echinococcus* by means of artificial immunization. The definitive host, the dog, was chosen as the experimental animal.

Two kinds of antigen were used in our attempts to immunize: (1) Scolices and the germinative membrane of *Echinococcus granulosus* were obtained from fresh fertile hydatid cysts of cattle. This material was dried in the incubator at 37°C., powdered and stored in bottles. Before use a 1% phenolized (0.5%) suspension was prepared. (2) Scolices, germinative membrane, and cuticular membrane from fertile and non-fertile hydatid cysts were obtained and prepared as was No. 1. There was no discrimination as to the kind or breed of dogs used. Young dogs weighing from 2.5 to 5.5 kg.

[1] Miller, H. M., Jr., *J. Prev. Med.*, 1931, **5**, 429.

were isolated and their stools examined for at least 5 consecutive days for the ova of taeniae. Ten dogs with a clean record were used as a preliminary series.

Eight dogs were immunized by 5 consecutive injections at 3-5 day intervals. The first dose was 0.5 cc. of the antigen suspension given subcutaneously; the subsequent doses were 1 cc. given intramuscularly. From 6 to 15 days after the last injection both the experimental and the control dogs were fed fresh fertile hydatid cysts. Except for 2 dogs which died of pneumonia before the last feeding, all dogs were fed at least 3 times before autopsy.

From 24 to 47 days after the first feeding and 10 days after the last feeding all dogs were sacrificed and autopsied. The intestines were thoroughly examined macroscopically and microscopically for evidence of *Taenia echinococcus* infestation.

Results. Macroscopic and microscopic examination of the intestines of the 2 control dogs showed that they were literally lined from the pylorus to the caecum with *T. echinococcus;* a count showed 1,384 taeniae per square centimeter.

Macroscopic examination of all the injected animals was negative; the intestines appeared perfectly normal.

Microscopic examination of the intestines of the injected dogs showed: 1 dog, entirely negative; in 1 dog a single *T. echinococcus* was found; 3 *T. echinococcus* were found in 2 dogs; 5 *T. echinococcus* were found in 1 dog; and 6 *T. echinococcus* were found in one other dog. The 2 animals which died 7 and 17 days respectively after the first feeding were entirely negative.

Conclusions. (1) It is possible to induce a marked degree of resistance to *Taenia echinococcus* infestation in dogs. (2) There was no appreciable difference between the efficacy of the 2 antigens used.

6599

Avitaminosis. XIV. Effect of Vitamin A Deficiency on Concentration of Blood Lipids of Albino Rat.*

BARNETT SURE, M. C. KIK AND ANNA E. CHURCH.

From the Departments of Agricultural Chemistry and Home Economics, University of Arkansas, Fayetteville.

Although it is well known that fats act as carriers of certain vitamins, nothing definitely has been established on the influence of avitaminosis on lipid metabolism. The literature is conflicting, because the difficulties involved in the analytical chemical methods are many. Another reason for inconsistent data, obtained on adequate control diets, is probably due to the fluctuating results produced by metabolic changes incident to the normal processes of digestion and absorption of lipids in the animal organism. In our lipid metabolism work we have fasted our animals one hour previous to bleeding. In order, however, to eliminate all possibilities of the influence of food on blood lipids, we are continuing our studies, fasting the rats for much longer periods. In the meantime, we feel that a summary of our results completed would be of interest to the clinician as well as to the nutritional investigator.

The dietary technique employed has been described in a previous communication.[1] The details of our blood methods will be submitted in a subsequent communication elsewhere.

The blood determinations were made once weekly and the experimental period lasted 50 to 65 days.

TABLE I.
Lipid Metabolism of Blood in Vitamin A Deficiency
(Fatty acids and phospholipids)
P—pathological; C—control.

Animal No.		Wt. gm.		Exp. Period	Fatty Acids (mg. per 100 cc.)		Phospholipids (mg. per 100 cc.)	
P	C	Init.	Final	(days)	Range	Ave.	Range	Ave.
♀ 8997		51	78	57	182-286	224	160-237	194
♀ 8801		47	70	51	192-253	227	181-229	204
	♀ 8798	46	167	57	190-266	211	171-234	194
	♀ 8800	48	155	41	192-246	218	170-213	194
♂ 8799		50	59	41	209-286	255	170-236	201
	♂ 8802	45	228	51	194-246	215	182-219	197
♂ 8803		60	77	58	169-253	213	170-213	189
	♂ 8804	58	225	58	185-274	225	179-236	196

*Research paper No. 271, Journal Series, University of Arkansas.

[1] Sure, B., Kik, M. C., and Walker, D. J., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 495.

For this study a total of 64 rats was employed, 27 controls and 37 pathological animals. In Tables I and II are submitted typical illustrations representative of the remainder of the group.

TABLE II.
Lipid Metabolism of Blood in Vitamin A Deficiency (Cholesterol).

Animal No.		Wt. gm.		Exp. Period	Cholesterol (mg. per 100 cc.)	
P	C	Init.	Final	(Days)	Range	Ave.
♀ 8821		85	84	30	69-100	82
	♀ 8822	90	155	30	70-104	89
♂ 8823		90	133	25	76-94	85
	♂ 8824	83	181	25	71-90	82
♂ 8825		67	93	29	71-106	80
	♂ 8826	60	180	29	68-94	81
♀ 8831		92	134	30	67-92	77
	♀ 8832	103	170	30	69-86	78

The pathological states of the animals during the advanced stages of the avitaminosis were associated with loss of weight, severe xerophthalmia and pneumonia, but as shown in Tables I and II the concentration of the blood fatty acids, cholesterol, and phospholipids fall within the same range as that of the controls, which made excellent growth without any apparent symptoms of vitamin A deficiency.

6600

Avitaminosis. XV. Effect of Vitamin D Deficiency on Concentration of Lipids of Blood of Albino Rat.*

BARNETT SURE, M. C. KIK AND ANNA E. CHURCH.

From the Departments of Agricultural Chemistry and Home Economics, University of Arkansas, Fayetteville.

In this investigation a total of 48 rats was employed, 16 controls and 32 pathological animals. The dietary technique has been described.[1]

The blood determinations were made once weekly and the experimental period lasted 30 to 40 days.

In Tables I and II are submitted typical illustrations representative of the remainder of the group.

* Research paper No. 272, Journal Series, University of Arkansas.

[1] Sure, B., and Kik, M. C., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 496.

TABLE I.
Lipid Metabolism of Blood in Vitamin D Deficiency
(Fatty Acids and Phospholipids)
P—pathological; C—control.

Animal No.		Wt. gm.		Exp. Period	Fatty Acids (mg. per 100 cc.)		Phospholipids (mg. per 100 cc.)	
P	C	Init.	Final	(Days)	Range	Ave.	Range	Ave.
♂ 8773		43	57	35	218-262	243	197-248	222
	♂ 8777	39	64	35	236-262	255	209-282	232
♂ 8775		49	61	35	228-262	247	197-253	227
	♂ 8790	46	60	33	214-279	252	209-260	237

TABLE II.
Lipid Metabolism of Blood in Vitamin D Deficiency (Cholesterol).

Animal No.		Wt. gm.		Exp. Period	Cholesterol (mg. per 100 cc.)	
P	C	Init.	Final	(Days)	Range	Ave.
♀ 8622		45	63	42	86-101	100
♀ 8623		45	58	42	82-101	93
♂ 8624		45	60	41	82-107	91
♂ 8625		45	64	41	88-107	94
	♀ 8626	42	69	41	88-112	100
	♂ 8627	35	59	41	82-92	8

It will be noted from the tabular data that the vitamin D deficient animals showed the same range of concentration of blood lipids as the controls.

Further work in progress will establish whether a longer fast will eliminate the changes in metabolism due to absorption of food lipids, and will bring about less fluctuations in the normal and whether, by the use of the modified technique, avitaminotic rats will show any definite departure from the normal in concentration of blood lipids.

6601

Dietary Requirements for Fertility and Lactation. XXV. Does Amount of Fat in Diet Influence Vitamin B Requirements for Lactation?*

BARNETT SURE.

From the Laboratory of Agricultural Chemistry, University of Arkansas, Fayetteville.

The experimental data presented by Evans and Lepkovsky[1] in support of the thesis that fat produces a sparing action on vitamin

* Research paper 297, Journal series, University of Arkansas.

[1] Evans, H. M., and Lepkovsky, S., *J. Biol. Chem.*, 1929, **83**, 269; *J. Nutri.*, 1931, **3**, 353; *J. Biol. Chem.*, 1932, **99**, 235.

B suggested an attempt to determine whether increasing amounts of fat in the ration would influence the incidence of infant mortality on a maternal diet deficient only in vitamin B or the vitamin B complex. The lactation efficiency index was studied on rations containing 3 levels of fat intake, namely 10, 20, and 30. The fat used was lard. Two types of yeast were used as a source of vitamin B complex for the control diets, *i. e.*, Northwestern dehydrated, and Fleischman's dried.† The same types of yeast, autoclaved, were used as a source of vitamin G for the pathological animals. Of the former 10% was used in the ration and of the latter, 15%. A total of 72 mothers with litters were employed. It was realized that, because of the large amounts of vitamin B required for lactation compared with that for growth, nothing like normal rearing of young could be anticipated by virtue of such a modification in the diet as the introduction of additional amounts of fat, since the basal rations were deficient in either the B vitamin or B vitamins. Yet, if large amounts of fat produce a sparing action on vitamin B requirements, the young should have been reared for a longer term before collapse ensued. The results, however, indicate no benefits derived in lactation from the additional increments of fat available to the nursing mother.

A critical examination of the data of Evans and Lepkovsky does not, in our opinion, justify the interpretation that fats have any sparing action on vitamin B requirements. We are, therefore, at present subjecting this problem to a severe test, using growing animals, the results of which will appear elsewhere in detail.

6602

Effects of Pitressin on Water Interchange in Normal and Decapitated Frogs.

F. R. STEGGERDA AND H. E. FREEDMAN. (Introduced by W. R. Amberson.)

From the Department of Physiology, University of Tennessee, Memphis.

Recently it was shown[1] that the permeability of the frog's skin is very much increased by injections of pitressin. Heller[2] also reported that frogs, previously decapitated, showed a smaller increase

† Supplied by the Standard Brands, Inc., New York.

[1] Steggerda, F. R., *Am. J. Physiol.*, 1931, **98**, 255.

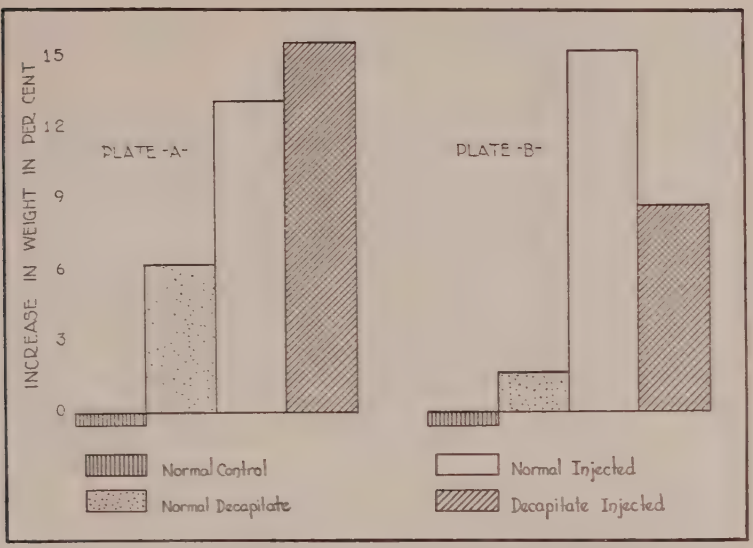
[2] Heller, J., *Arch. f. exp. Path. u. Pharm.*, 1930, **157**, 298.

in weight than normal frogs after pitressin injections. Since Adolph[3] and others have reported pronounced increases in water uptake after pithing, it was of interest to repeat some of Heller's experiments with special reference to the effects of pitressin on decapitated frogs at various intervals of time after the decapitation. All of Heller's experiments were performed from 4 to 64 hours after decapitation. Our experiments can be separated into 2 groups—those done immediately after decapitation and from 26 to 48 hours after decapitation.

A complete experiment consisted in placing 4 normal frogs (30-40 gm.) in a glass container with enough water to nearly submerge them. The temperature was kept slightly below room temperature by the occasional addition of ice cubes. After weighing each frog accurately to 0.1 gm., on a triple-beam balance in a previously weighed container, 2 were decapitated according to the technique of Heller, which consists of placing one blade of the open scissors in the frog's mouth and making a sharp cut across the head at the level of the ears. The loosened head was then held in place by 2 stitches, which assisted in preventing severe hemorrhage. Pitressin (Parke-Davis) was then injected into the dorsal lymph sac of a normal and decapitated frog (0.1 cc. per 10 gm. body weight). The remaining 2, one a normal and another decapitated frog were kept as controls in the same container. Weighings were made at half hour intervals for a period of 6 hours. Six such experiments were carried out immediately after decapitation, and 6 others from 26 to 48 hours after decapitation. The accompanying graph represents the average of the results obtained in these two series. Plate A shows the effect of pitressin on the water interchange immediately after decapitation, while plate B shows the effect 26 to 48 hours later.

The normal control remains quite the same in both cases; but the normal decapitate shows a considerable increase in weight immediately after decapitation; whereas the rate of increase is very much less when a certain period of time has elapsed between decapitation and the time of the experiment (see plate B). Likewise, the rate of increase in the decapitated injected frog immediately after the operation is more pronounced than that of the control injected frog, and similarly when observations are made from 26 to 48 hours after the operation the weight increase of the decapitated frog is less than that of the control injected.

[3] Adolph, E. F., *Am. J. Physiol.*, 1931, **96**, 569.



Although our results as indicated in plate B are nearly a duplicate of the results of Heller, our interpretation is quite the opposite of his. He asserts that decapitation decreases skin permeability to water, while our results indicate that decapitation increases skin permeability to water, and that the reason for the decrease in water uptake resulting from pitressin injections after a certain period of time, is that the frogs have already reached a state of edema.

Although these experiments do not offer much information as to the exact function of pitressin in its relation to skin permeability, we feel that the immediate results of decapitation show that the brain may serve as a regulatory mechanism for skin permeability.

6603

Vagus Stimulation and Rate Changes in the Turtle Heart.

A. S. GILSON, JR.

From the Department of Physiology, Washington University School of Medicine, St. Louis.

Observations previously reported[1] showed that stimulation of either vagus nerve of a turtle might be adjusted to produce some slowing of the rate of the sinus beat although only a part, if any, of

[1] Gilson, A. S., and Irvine-Jones, E., *Am. J. Physiol.*, 1929, **90**, 361.

the sinus impulses were conducted to the atria and ventricle. Further investigation of vagus stimulation in producing these phenomena are presented here.

In many preparations either the right or left vagus may be stimulated to produce atrial inotropic depression of the atria with no chronotropic effects. Only rarely is it impossible to do this by stimulation of the left vagus. With the right vagus the separation of effects is rarely as pronounced as with the left.

If right vagus stimulation of strength just sufficient to produce definite chronotropic changes be used, continued stimulation usually produces inotropic depression of the sinus and slowing to a rate somewhat more than half the normal rate. Frequently the pacemaker, normally on the right side, then shifts to an abnormal locus, usually to a region in the left side of the sinus symmetrical with the normal pacemaker. This beat may be propagated so that the atria and ventricle respond regularly or with occasional dropped beats. With somewhat stronger stimulation the aberrant pacemaker may continue active but sino-atrial block prevents propagation to the atria and ventricle.

With left vagus stimulation, the interpretation of records because of the aberrant pacemaker is avoided and the chronotropic effect is much reduced. In most preparations, the left vagus contributes but few fibers to the normal pacemaker region. Evidence for this is, for example, the fact that in the majority of preparations, low stimulation rates (ca. 5 per sec.) produce little or no slowing of the heart and no visible inotropic depression of the sinus at any strength of stimulation used. With more rapid stimulation rates there occurs summation of effect and the heart may be slowed and stopped. With properly adjusted strength and rate the heart may be kept for considerable time in a state such that there is a regular sinus beat, somewhat slower than normal and a fractionate response of the atria and ventricle. This fractionation is due to an extremely long sino-atrial conduction time and consequent functional block.

If the heart be divided by a median sagittal cut, the right and left sides will each beat rhythmically, each side being driven by its own pacemaker. All crossed vagus effects are removed. Under these conditions stimulation of either vagus will cause depression of the corresponding side of the heart, the distribution of effects being similar on the two sides. Frequently an atrial inotropic effect may be obtained without change of rate. Sinus inotropic effect and chronotropic effects on either side appear at nearly the same

time though usually there is depression of strength of the sinus contraction before there is measurable slowing of rate.

By one method or another, it is usually possible to produce atrial inotropic effects in the turtle heart without accompanying chronotropic effects. The usual interpretation placed on the Engelmann hypothesis is that the "chronotropic fibers" produce only chronotropic effects, while "inotropic fibers" produce only inotropic effects. Our experiments on the turtle heart do not exclude the possibility that the sinus venosus and the pacemaker within the sinus are innervated by inotropic and chronotropic fibers of different function and differing from each other either by number or by threshold. Using the heart as our only indicator, however, we have not been able to find any consistent method of differentiating fibers causing sinus inotropic effects from fibers causing slowing of the pacemaker.

6604

Change in Viscosity of Mucin with pH.

C. O. MILLER AND JEAN M. DUNBAR. (Introduced by C. J. Farmer.)

From the Department of Chemistry, Northwestern University Medical School.

For many years physiologists have known that mucin plays a lubricating, protective, and soothing rôle in gastro-intestinal function. It is synthesized in the mucous cells and is the characteristic constituent of mucus. The slimy, viscid mucus spreads over the surface of the mucosa and facilitates the passage of food particles over the mucosa with minimum of injury.

Fogelson[1] and Atkinson[2] have shown that when patients with peptic ulcer are given mucin, there is prompt relief of the subjective symptoms without recurrence in most patients for as long as one year.

Since mucin is a physiological secretory product of the gastro-intestinal tract and since mucin administered orally to ulcer patients gives every indication of healing, it suggests that the metabolic difficulty leading to ulcer formation may be a disturbance of mucin metabolism.

[1] Fogelson, S. J., *J. Am. Med. Assn.*, 1931, **96**, 673.

[2] Atkinson, A. J., *J. Am. Med. Assn.*, 1932, **98**, 1153.

This is the first of a series of papers on the study of mucin metabolism, its importance in the functioning of mucous membrane, and its probable bearing upon ulcer formation.

Fogelson[1] and Atkinson[2] have pointed out that mucin has a high acid combining power and thus helps to control gastric acidity. Jones and Ivy[3] have shown that the buffer action of mucin is less than the buffer action of peptones. Since peptones are not peculiarly beneficial in ulcer therapy, they conclude that control of gastric acidity is not the important beneficial factor in mucin therapy.

It is obvious that the more viscous and tenacious the mucin is, the more effective it will be in protecting the mucosa. We have studied the change in viscosity of mucin with pH at the temperatures 25° and 37°C.

We used Wilson's Prepared Gastric Mucin, prepared by the method of Fogelson. The samples* were made up by incorporating phosphate buffer mixtures, sodium hydroxide, or 0.1 N hydrochloric acid to portions of isoelectric gastric mucin in such proportions that they gave emulsions varying in pH from 2 to 8. The pHs were determined by the quinhydrone electrode. Five percent emulsions were made up by adding water to 10 gm. samples of mucin and making them up to 200 cc. Care was always exercised to insure that all of the mucin was dispersed in the water. The emulsions were allowed to flow from a 100 cc. capillary pipette, kept at a constant temperature. The time of flow was recorded. The time of flow for water was obtained for comparison. When the time of flow is plotted against pH, it is found that the viscosity is at a maximum at the isoelectric point, 4.98. In this respect, mucin differs from proteins in general in that they show their minimum viscosity at their isoelectric point. The time of flow of a 5% emulsion of mucin at a pH of 4.98 at 37°C. is approximately 2½ times greater than the time of flow at a pH of 7.4, and the time of flow at a pH of 2 is 1½ times greater than at a pH of 7.4. We were interested in relative rates of flow and not absolute viscosity; hence we have not calculated viscosities in absolute units. It is significant that mucin shows its maximum viscosity at its isoelectric point, for it indicates that mucin has a greater protective action upon gastric mucosa in the presence of hydrochloric acid than it has in its absence.

In view of this data, we may visualize the protective action of mucin on the surface of the cells of the mucosa in microscopic

[3] Jones, K. K., and Ivy, A. C., PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 218.

* We are indebted to Dr. David Klein of the Wilson Laboratories for these samples.

dimensions as follows: Mucin is synthesized by the mucous cells at approximately pH 7.4 and presumably is secreted in the mucus at a pH of 7.4. Ivy and Oyama[4] found that mucus from pouches of the pyloric antrum of dogs had a pH of 7.0 to 7.5. The mucus spreads over the surface of the cell and the adjacent cells in a microscopic layer. When the gastric juice comes in contact with the alkali mucinate, isoelectric and acid mucin is formed. The viscosity of this mixture will depend upon the relative amounts of alkali mucinate, isoelectric mucin, and acid mucin present. Whatever the proportions may be, the viscosity will be greater than it was at the time of secretion. The increase in viscosity further retards the diffusion of hydrochloric acid and pepsin through the layer so that there is a progressive increase in pH towards the surface of the cell. The continual secretion of mucus maintains a pH very near 7.4 at the surface of the cell. This offers a probable explanation why the stomach does not digest itself. When a petechial injury to the mucosa occurs, the large excess of tenacious mucin slips over the injured area and protects it from the action of gastric juice. This may explain the marked healing ability of the mucosa. The outer layer of mucus is continually being rubbed off by the food particles sliding over the surface. A part of it may coat these particles with the result that they slide over the mucosa with less trauma. It becomes mixed with the gastric contents and passes into the intestine.

If we have described correctly the events that take place in the thin mucus layer on the surface of the cells lining the digestive tract, it is evident that in a condition in which there is a disturbance of mucin metabolism, there is less protection for the cells against the corrosive action of gastric juice. When an injury occurs, there is less mucus available to cover the injury and to protect it from the corrosive action of the gastric juice.

We have shown that the viscosity of gastric mucin changes with pH, passing through a maximum at its isoelectric point, 4.98. We have suggested the importance of mucin metabolism in the functioning of the mucosa of the digestive tract and that a disturbance in the level of mucin metabolism may be a predisposing cause of ulcer formation.

Further investigations on other conditions which affect the viscosity of mucin are in progress.

---

[4] Ivy, A. C., and Oyama, Yutaka, *Am. J. Physiol.*, 1921, **57**, 51.

6605

Origin of Glucuronic Acid in the Urine of Rabbits.

C. O. MILLER AND J. A. CONNER. (Introduced by C. J. Farmer.)

From the Department of Chemistry, Northwestern University Medical School.

Two theories have been proposed for the origin of glucuronic acid in the urine. Sundwick[1] and Fisher and Piloty[2] suggested that the toxic substance was conjugated with glucose by a glucoside linkage, followed by the oxidation of the terminal alcohol group to a carboxyl group. A second theory[3] states that the body is able to synthesize glucuronic acid from amino acid metabolites as it needs glucuronic acid for detoxification. Quick[4] thinks that the animal body is able to detoxicate itself in both ways. He[5] has reviewed the literature on the subject. One must conclude from reviewing the subject that the results of the investigations are inconclusive.

Glucuronic acid exists preformed in the body, in mucin. It is continuously being digested in the intestine, and its constituents are absorbed and restored to the body. It is reasonable to think that glucuronic acid would be one of the digestion products and that it is a normal constituent of the blood stream. It seemed to us that the glucuronic acid arising from mucin metabolism would be available for use by the body in detoxication when needed and that it is unnecessary to assume that the glucuronic acid appearing in the urine as conjugated glucuronic acids arises from the oxidation of carbohydrates or by synthesis from amino acid metabolites. Our experiments were carried out to see if mucin feeding had any significant effect upon the conjugation of glucuronic acid as indicated by the quantity excreted.

A group of 11 rabbits was fed a diet of oats, carrots, and water and each given 2 gm. of menthol in water by stomach tube for 3 days. Their average daily excretion of menthol glucuronic acid calculated as glucuronic acid was 0.41 gm. Glucuronic acid was determined by the method of Quick.[6] The rabbits were then given water and fasted for 5 days. They were each given 2 gm. of menthol in water by stomach tube. They showed signs of menthol intox-

[1] Sundwick, E., *Akadem Abhandlungen*, Helsingfors, 1886.

[2] Fischer, E., and Piloty, G., *Ber. Chem. Ges.*, 1891, **24**, 522.

[3] Lusk, Science of Nutrition, Saunders, 1928, 683.

[4] Quick, A. J., *J. Biol. Chem.*, 1926, **70**, 397.

[5] Quick, A. J., *J. Biol. Chem.*, 1924, **61**, 679; 1926, **70**, 59, 397.

[6] Quick, A. J., *J. Biol. Chem.*, 1924, **61**, 667.

ication during this period. During the third period of 3 days, they were each given water and fed 20 gm. of dextrin and 2 gm. of menthol by stomach tube. During this period, the average daily excretion of glucuronic acid was 0.25 gm. The quantity excreted decreased from day to day. The symptoms of toxicity increased during dextrin feeding. We interpret the difference in levels of average daily glucuronic acid excretion on a normal diet and a dextrin diet, and the increasing rather than decreasing symptoms of toxicity to indicate that carbohydrates are either not the precursors of glucuronic acid in the urine, or the rate of synthesis is too slow to protect rabbits adequately. This is in accord with the observations of other investigators.

A second group of 6 rabbits was used to determine whether mucin or proteins, like milk proteins, would have any effect upon glucuronic acid excretion. Five of the rabbits were 8 months old, and from one litter. The sixth was full grown. They were fed a commercial feed in pellet form, recommended as a balanced ration for rabbits, and 2 gm. menthol in water by stomach tube daily, throughout the experiment. During the first 4 days the average daily excretion dropped progressively. During the next 4 days, they were each given 2 gm. mucin in water by stomach tube. During this period, the average daily excretion increased progressively. There were no symptoms of toxicity. During the last period, they were given milk to drink instead of water. Each rabbit drank about 200 cc. daily. The average daily excretion of glucuronic acid dropped progressively, and they developed marked symptoms of intoxication.

We interpret this to mean that the glucuronic acid obtained in the digestion of mucin is readily available for detoxication purposes. Since there was a progressive decrease in glucuronic acid excretion during the first period when rabbits were given a balanced ration and since milk did not prevent them from developing symptoms of intoxication, we question whether the body can synthesize glucuronic acid from either amino acid metabolites or carbohydrates. At least, it indicates that the rate of synthesis is so slow that it does not prevent the development of symptoms of intoxication. Mandel and Jackson[7] found that feeding meat to fasting dogs caused an increase in the excretion of camphorol glucuronic acid. This has been interpreted as evidence of the ability of the body to synthesize glucuronic acid from amino acid metabolites. Since connective tissue of meat contains preformed glucuronic acid, it seems to us that the in-

[7] Mandel and Jackson, *Am. J. Physiol.*, 1902, **8**, XIII.

crease in camphorol glucuronic acid excretion was due to the availability of preformed glucuronic acid from the digestion of the meat and not to synthesis from amino acid metabolites.

Fromm and Clemens[8] state that a strong rabbit can tolerate 5 gm. of menthol daily. Our experience does not confirm this. We used young rabbits. The toxicity of menthol may decrease with age. Biberfeld[9] states that a rabbit can tolerate 2 gm. of menthol daily if it is fed greens. Our young rabbits could not tolerate 2 gm. of menthol on a diet that did not contain greens. This suggests to us that the greens may either contain preformed glucuronic acid or contain substances from which rabbits can readily obtain glucuronic acid. This also indicates that either the body is unable to synthesize glucuronic acid or that the synthesis proceeds slowly.

A third group of 2 rabbits was given water and fasted for 6 days. They were then given 2 gm. of menthol daily by stomach tube for 3 successive days. Their average daily excretion of glucuronic acid dropped from 0.96 gm. to 0 gm. in the 3 days. This indicates that the rabbit after a 6-day fast still contains glucuronic acid available for conjugation. The rate of decrease in menthol glucuronic acid excretion of fasting rabbits was faster than for rabbits that were well fed. The excretion of menthol glucuronic acid in rabbits receiving food never reached 0. The rate of decrease of menthol glucuronic acid may give some indication of the amount of mucin in the body. After the 3 days' fasting and menthol administration they were fed pellets which we had previously found did not protect rabbits from a decrease in glucuronic acid excretion, and 2 gm. of mucin per day, but no menthol, for 4 days. At the end of 4 days, they were given 2 gm. menthol each for 2 days. Their average daily excretion of glucuronic acid as menthol glucuronic acid was 0.53 gm. for the first day and 0.42 gm. for the second. This indicates that during the 4-day period of mucin feeding either glucuronic acid was stored in the body or more glucuronic acid already in the body was made available for conjugation. We think that the dietary mucin was the chief factor in raising the level of menthol glucuronic acid excretion.

We conclude from our experiments that glucuronic acid is liberated in the digestion of mucin and that it is readily available for conjugation with certain toxic substances when needed by the body. It seems unnecessary to us to assume that the detoxication mechanism necessitates the synthesis of glucuronic acid from carbohydrates

[8] Fromm, E., and Clemens, P., *Z. Physiol. Chem.*, 1901, **34**, 385.

[9] Biberfeld, J., *Biochem. Z.*, 1914, **65**, 479.

or amino acid metabolites. Our findings lead us to doubt if the rabbit can synthesize glucuronic acid from carbohydrates or amino acid metabolites.

Further investigations are in progress on the ways by which the body loses glucuronic acid and the importance of it in the animal economy.

6606

Studies on the Metabolism of Glucuronic Acid in the Dog.

C. O. MILLER, F. G. BRAZDA AND E. C. ELLIOT.
(Introduced by C. J. Farmer.)

From the Department of Chemistry, Northwestern University Medical School.

Since glucuronic acid and glucosamine are characteristic constituents of the prosthetic group of mucin, the biochemical synthesis of mucin can not take place without them. Very little is known about the metabolism of these substances, except that they are probably not glycogenic and are surprisingly inert in the body. We are interested in studying the metabolism of these substances because their metabolism is so closely related to the metabolism of mucin and gastro-intestinal function.

We wish to report our findings to date. When dogs are fed borneol, it is excreted as borneol glucuronic acid. In another paper of this series, Miller and Conner show that the glucuronic acid arising from the digestion of mucin in rabbits is readily available for conjugation with menthol, and that either the rabbit is unable to synthesize glucuronic acid from carbohydrates or amino acid metabolites, or that the synthesis takes place slowly. We were interested in determining if this applied to dogs.

Three dogs having an average weight of 9.5 kg. were fed a stock diet of bread and corn meal gruel containing a little meat broth. They were given 5 gm. of borneol in 90 cc. of 1% agar by stomach tube for 31 successive days. Their average total excretion of glucuronic acid as borneol glucuronic acid was 30.78 gm. Their average daily excretion of glucuronic acid was 1.11 gm.* At the end of the experiment their average weight was 8.12 kg. During

* Glucuronic acid was determined by the method of Quick, *J. Biol. Chem.*, 1931, **61**, 667.

the last 8 days the average daily excretion was 0.63 gm., which is lower than the average daily excretion for the entire period. The dogs were then fasted for 5 days, but the daily administration of borneol was continued. The average daily excretion for the 5-day period was 1.06 gm., an increase of 68% over the average daily excretion during the previous 8-day period. They were then given 10 gm. mucin and 5 gm. borneol daily, but no food, for 7 days. The average daily excretion of glucuronic acid during this period increased to 1.43 gm., an increase of 127% over the 8-day period. We then fed them the stock diet and stopped feeding borneol. The excretion of borneol glucuronic acid was negligible after 48 hours. The dogs apparently suffered no ill effects from the prolonged administration of borneol.

We interpret these findings to indicate that glucuronic acid obtained from the digestion of mucin is readily available for conjugation with borneol in the dog; that fasting dogs can conjugate glucuronic acid more readily than dogs given adequate food, due probably to the accelerated endogenous catabolism during starvation; that dogs either have stored in their bodies a considerable quantity of glucuronic acid, probably as a deposit protein in addition to their supply of mucin, or they can synthesize it from carbohydrates and amino acid metabolites; the loss of weight on an adequate diet makes us believe that the former view is more nearly correct; that dogs can excrete borneol glucuronic acid continuously for 43 days without causing any profound ill effects; that the efficiency of conjugation is dependent directly upon the total amount of glucuronic acid available, decreasing progressively with glucuronic acid availability, since the average daily excretion for the last 8-day period was less than the average daily excretion for the entire 31-day period.

Another group of 3 dogs having an average weight of 16 kg. was fed the stock diet and 10 gm. of mucin per day. The dogs were started on 5 gm. of borneol per day. The dosage was slowly increased until after 2 months, they were receiving 19-25 gm. of borneol per day, which was 1.3 gm. per kg. body weight. It is stated that dogs can tolerate 5 gm. of borneol per day without ill effects, the inference being that a quantity appreciably larger than this causes toxic symptoms to appear. Mucin was added to the diet with the expectation that it would protect the dogs against the toxic effects of large doses of borneol. During the third month, the dogs were fed 1.3 gm. borneol per kg. body weight for 24 days. The average daily excretion of glucuronic acid was 6.23 gm. Mucin

was then withdrawn from the diet. One dog had contracted distemper and was killed 2 days later. The daily excretion of glucuronic acid for the remaining 2 dogs continued at approximately the previous level. After 17 days, a second dog died, following a profound drop in glucuronic acid excretion. Death was probably due to the toxic effect of large doses of borneol without the protecting effect of mucin. After 21 days the third dog was fasted. The average daily excretion continued high for 7 days, when suddenly the glucuronic acid excretion dropped, the dog became toxic, and died within 3 days.

We interpret the results of these experiments to indicate that mucin protects against large doses of borneol because it supplies glucuronic acid to the organism; that the increase in average daily excretion over dogs not receiving mucin is due to the mucin itself; that the body is able to store a considerable quantity of glucuronic acid, probably as mucin or as a deposit protein, which can act as a source of glucuronic acid when needed; that the organism can be more or less depleted of this supply, and when this occurs, toxic symptoms appear.

A third group of 5 dogs was fasted. They were fed 5 gm. of borneol daily. Three dogs showed a progressive rise of about 400% in excretion of glucuronic acid in 2 weeks. One dog was pregnant and died after 2 weeks' fasting. Autopsy revealed a large prepyloric ulcer, 2 duodenal ulcers, and multiple ulcers in the pylorus. The areas involved were about ½ inch in diameter. A second dog became very weak and somewhat toxic after 14 days. It was fed 25 gm. mucin daily. During the remainder of the 37-day fasting period, glucuronic acid excretion dropped slightly and then remained constant. The third dog after the 20th day of fast showed a progressive drop in glucuronic acid excretion until the 34th day, after which there was a rise for 3 days.

The other 2 dogs of this group were fasted but given 10 gm. mucin daily for 37 days. There was a progressive rise in the glucuronic acid throughout the entire fasting period from an average daily excretion for the first 5 day period of 1.5 gm. to 2.96 gm. for the last 5-day period.

The glucuronic acid which is excreted during fasting may come either from the synthesis of glucuronic acid, from carbohydrates or amino acid metabolites, or from the metabolism of deposit protein in the accelerated endogenous catabolism. When fasting dogs are fed mucin there is a continual rise in excretion which lasts as long as 37 days. This indicates again that glucuronic acid arises from

mucin. If our previous hypothesis is correct, that the efficiency of glucuronic acid excretion is dependent upon the total amount of glucuronic acid available, then the progressive rise in glucuronic acid excretion during starvation when 10 gm. of mucin is fed indicates that glucuronic acid can be stored during fasting in some form and hence is not oxidized in the body for energy.

The autopsies on these dogs were made by Dr. S. J. Fogelson. With few exceptions all dogs that had excreted a large amount of glucuronic acid, showed a gastritis more marked in the pyloric area than in the fundus, and a marked duodenitis. The gastric mucosa was slimy, indicating that some mucin was still present. In one case, where a female was fasted for 2 weeks, one prepyloric, 2 duodenal ulcers, and multiple ulcers of the pylorus were found. We think that the gastritis and duodenitis result from the interference in the normal mucin metabolism of the mucosa incident to the loss of glucuronic acid. While it is possible that borneol may have an irritating action directly upon the mucosa and produce the gastritis and duodenitis, it seems that the feeding of borneol to dogs for over 100 days, during which time massive doses were fed for 40 days, would produce more ill effects than were noticed. Investigations on Pavlov pouch dogs bear out our conclusion.

Many more animal experiments must be performed before any final conclusions can be formed on the metabolism of glucuronic acid. We submit our findings and our tentative conclusions.

We are deeply indebted to Dr. S. J. Fogelson for valuable assistance in this investigation.

6607

Glucuronic Acid as a Growth Factor in Guinea Pigs.

C. O. MILLER, A. E. SIEHRS AND F. G. BRAZDA.
(Introduced by C. J. Farmer.)

From the Department of Chemistry, Northwestern University Medical School.

It is generally considered that the animal body can synthesize glucuronic acid either from carbohydrates or from amino acid metabolites. Miller and Conner have reported results previously which indicate that either the rabbit cannot synthesize glucuronic acid or that the rate of synthesis is low. A similar investigation on dogs did not give such definite indications. In comparing the chemical properties of glucuronic acid with the chemical properties of Vitamin

C, we were struck with the close similarity existing between them. Svirbely and Szent-Györgyi[1] have reported evidence indicating that Vitamin C is an hexuronic acid. Glucuronic acid is an hexuronic acid and we decided to compare its biochemical properties with those of Vitamin C, even though Hirst and Reynolds[2] state that the hexuronic acid isolated by Svirbely and Szent-Györgyi is not glucuronic acid. We were interested also in the importance of glucuronic acid in growth.

If glucuronic acid cannot be synthesized by the growing animal, it would then be an essential carbohydrate acid for growth, for it is a constituent of the glycoproteins in cartilage, connective tissue and mucin. If glucuronic acid can be synthesized by the growing animal, then they should experience no difficulty in growth on a diet deficient in glucuronic acid.

Eighteen guinea pigs weighing 280-320 gm. were fed the basal Vitamin C deficient diet of Sherman, LaMer, and Campbell,[3] *ad lib.* Of these, 6 were fed, by dropper, a water solution containing 2 mg. of an equilibrium mixture of glucuronic acid* and its lactone, for 9 days, and thereafter 10 mg. per day. Six were fed ¼ gm. of gastric mucin per day, mixed with dry feed, and 6 were kept as controls. The control animals began to lose weight after the second day. The group receiving mucin gained an average of 2½ gm. during a period of 5 days, when they started to lose. The group receiving glucuronic acid gained 12 gm. during a period of 8 days, after which they began to lose weight. By the 14th day, all of the animals had developed symptoms of scurvy. No differences between the groups as to severity were noticeable. The rate of loss of weight of the groups from the 10th to 14th day was practically the same. On the 14th day, each was given 1.7 cc. of orange juice and 1/6 of a medium-sized orange. The glucuronic acid and mucin groups started to gain weight immediately, but the control group continued to lose weight for another day. One of the control group died on the 14th day and 2 more on the 16th. Autopsy showed symptoms of scurvy. The cortex of the adrenals of the animal that died on the 14th day did not reduce silver nitrate. On the 16th day 1 glucuronic acid pig died.

[1] Svirbely, J. L., and Szent-Györgyi, A., *Nature*, 1931, **129**, 576.

[2] Hirst, E. L., and Reynolds, R. J. W., *Nature*, 1931, **129**, 576.

[3] Sherman, H. C., LaMer, H. L., and Campbell, H. L., *J. Am. Chem. Soc.*, 1922, **44**, 165.

* The glucuronic acid was prepared by the method of Quick, *J. Biol. Chem.*, 1927, **74**, 331.

On the 30th day, the control group were 10 gm. below their original weight whereas the mucin group had gained 7 gm. and the pigs receiving glucuronic acid had gained 36 gm. There were no external symptoms of scurvy. One animal was killed from each group. Autopsy showed slight hemorrhagic areas around the joints. The cortices of the adrenals reduced silver nitrate. Orange juice was removed from the diet with the exception of one pig of the glucuronic acid group. On the 31st day, the weight of the control group reached its original level, but they started to lose weight rapidly. The mucin group started to lose weight slowly on the 32nd day, and the pigs receiving glucuronic acid started to lose weight rapidly on the 34th. Again, mucin and glucuronic acid delayed the onset of scurvy on a Vitamin C deficient diet.

We conclude from our experiments that Vitamin C is not glucuronic acid since it does not protect guinea pigs on a scorbutic diet from developing scurvy nor alter the severity of the condition of scurvy. Our data indicate that the addition of glucuronic acid to the diet we fed was beneficial to the guinea pig, since it postponed the period of loss of weight in the onset of scurvy and facilitated weight recovery when orange juice was added to the diet. Since there are no data in the glucuronic acid content of foods, we do not know how much may be present in the food.

Since the guinea pigs which were fed glucuronic acid gained weight faster than the control group, we consider this as evidence indicating that the guinea pig either cannot synthesize glucuronic acid or that the synthesis proceeds slowly. Also we have observed that the adrenals of a guinea pig which has died as a result of scurvy do not reduce silver nitrate solution.

6608

Electrical Currents Associated with Sound Reception by the Ear.

T. H. BAST, R. NOER, ROBERT WEST, O. L. BACKUS, M. KRASNO AND J. A. E. EYSTER.

From the Departments of Anatomy, Speech and Physiology, University of Wisconsin.

The remarkable phenomenon of the reproduction of voice sounds and musical tones entering the cat's ear, by amplification of electrical currents from the eighth nerve and neighboring structures,

observed by Wever and Bray,[1] has been confirmed by Davis and Saul,[2] Adrian,[3] Hughson and Crowe[4] and by Witting.[5]* If the view held by the discoverers of this phenomenon is substantiated, that these currents represent true action currents related to hearing, it will necessitate a marked revision of our present views in regard to the refractory period and frequency of impulse transmission in sensory nerves, or else the assumption of an elaborate coordinating mechanism.

We have found the same phenomenon in the dog under sodium barbital or nembutal anesthesia. Using a 6 stage amplifier, sufficient volume is obtained to make clear records on a phonographic recorder. Words, sentences and single frequencies between 100 and more than 5000 per second are clearly reproduced. Our results, in part confirming, in part supplementing, and in part contrary to previous observations may be summarized as follows:

1. In common with previous workers we find that the phenomenon may be adduced by leads not only from the eighth nerve but also over a rather large contiguous region and in the neighborhood of the cochlea. With one electrode on the cut surface of muscle several centimeters away from the nerve, we have obtained positive results of varying degree of intensity with the other electrode (a) on the eighth nerve, (b) on the tenth nerve, (c) near the round window within the bulla (Adrian), (d) on the neighboring cerebellar cortex, (e) on the bony tentorium, (f) on the neighboring cerebral cortex, (g) within the external auditory meatus, and finally (h) on the occipital bone. Leads (c) and (g) may be obtained without opening the skull and give satisfactory volume and clarity of reproduction. Negative results were obtained with the electrode on the parietal bone. While the volume of sound obtained at a given amplification is as a rule greater when the eighth nerve is in the circuit, clarity of reproduction of the speaking voice is usually greater when

[1] Wever, E. G., and Bray, C. W., *Science*, 1930, **71**, 215; *Psych. Rev.*, 1930, **37**, 365; *Proc. Nat. Acad. Science*, 1930, **16**, (5), 344.

[2] Davis, H., and Saul, L. J., *Proc. Am. Physiol. Soc.*, 1932, 28; *Science*, 1931, **74**, 205.

[3] Adrian, E. A., *J. Physiol.*, 1931, **71**, 28.

[4] Hughson, W., and Crowe, S. J., *J. Am. Med. Assn.*, 1931, **91**, 2027.

[5] Witting, E. G., *Laryngoscope*, 1932, **42**, 497.

* Kruzer and Dorge[6] fail to elicit the phenomena and ascribe the result to fortuitous circumstances connected with the apparatus. This explanation seems to be eliminated by the precautions used by Wever and Bray and subsequent workers to exclude the possibility of extraneous sources of current.

[6] Kruzer, G., and Darge, H., *Science*, 1932, **75**, 105.

the connection is made with neighboring structures. It is suggested that this may be due to interference in the former case brought about by action currents of lower frequency arising from the cochlea and conducted through the auditory nerve.

2. Placing pure chloroform in the open wound so that the eighth and tenth nerve lie in a pool of this substance does not decrease the volume obtained from the eighth nerve but tends to clarify reproduction. The same is true of 4% cocaine. Wever and Bray and Adrian have been unable to abolish the phenomenon by similar nerve depressants applied to the nerve, although Davis and Saul found evidence of depression with novocaine along the central auditory pathways. Pure chloroform applied locally to the sciatic nerve in the frog abolishes sensory action currents in 2 minutes or less.

3. The phenomenon does not always disappear shortly after cessation of the vascular circulation.[1,2] In animals dying from hemorrhage, fairly clear reproduction may be obtained for more than an hour after cessation of the heart beat, and intelligible phonographic records made as late as three-quarters of an hour after cardiac death. There is a rapid decrease in intensity within the first 5 minutes, and then a very slow decrease until final disappearance. In animals killed by the intravenous injection of a rapidly acting poison (chloroform, magnesium chloride), the phenomenon may disappear completely within 3 to 8 minutes.

4. While we have not as yet measured the magnitude of the potential changes associated with this phenomenon, there is clear evidence that this is much smaller than that associated with known sensory action currents.

5. In contrast with the rather wide area over which these currents may be obtained, it is impossible with our apparatus to detect action currents from other sensory nerves, unless one or both of the leads makes direct contact with the nerve.

6. If these currents arise from microphonic action of the cochlea, as suggested by Adrian, the apparatus is not a simple resistance microphone, since a condenser in the input circuit to the amplifier, reducing the small constant grid current to zero, is without effect. We have some evidence that the underlying process may be of the nature of streaming potential.

The origin of these currents, and their relation if any to hearing, requires further investigation.

6609

Gonadotropic Activity of the Pituitaries of Horses.*

ARTHUR A. HELLBAUM. (Introduced by Frederick L. Hisaw.)

From the Department of Zoology, University of Wisconsin.

There is a quantitative difference in the relative gonadotropic capacity of the pituitaries of various types of animals. The pituitaries of hogs and sheep contain much more of the gonad stimulating substances than do the pituitaries of beef.[1] According to Wallen-Lawrence and Van Dyke[2] similarly prepared extracts of beef and sheep glands showed the former to have less than one-tenth the activity of the latter.

During a series of studies of horse pituitaries collected at various times of the sexual cycle, and at different periods in pregnancy, we have compared their gonad stimulating activity with that of the pituitaries of other animals. The horse glands have considerably more gonadotropic activity than those of sheep, which have been the chief source of material for comparison.

The material was prepared by grinding the fresh glands, desiccating them in 2 volumes of acetone 3 times, completely drying in a warm current of air, and powdering.† A typical experiment is as follows: A total subcutaneous injection of 12.5 mg. of the dried powder administered twice daily over a period of 5 days to 21-22 day old rats produced ovaries weighing 97.4, 64.6, 35.0, 51.2, 35.4, 79.3, and 51.1 mg., average 59.1 mg.; 25 mg. total injection resulted in ovarian weight of 192, 107, 263.4, average 187.5 mg.; and 50 mg. produced ovaries 324.3, 369.2, 250.7, and 177.9 mg. respectively, averaging 280.5 mg.

Ovaries of rats injected with 50 mg. of dried sheep pituitary powder weighed 35-50 mg., while the ovaries of normal untreated controls weighed 12-14 mg. It is thus evident that the concentration of the sex stimulating hormones in horse pituitaries is approximately 4 times that of similarly prepared sheep material.

Furthermore, the effect of horse pituitary powder on the ovaries

* Aided by a grant from the National Research Council, Committee on Problems of Sex, and the University of Wisconsin Research Fund, administered by Frederick L. Hisaw.

[1] Bugbee, E. P., Simond, A. E., and Grimes, H. M., *Endocrinol.*, 1931, **15**, 41.

[2] Wallen-Lawrence, Zonja, and Van Dyke, H. B., *J. Pharm. and Therap.*, 1931, **43**, 93.

† The horse pituitaries were kindly furnished by the Chappel Bros., Inc., of Rockford, Ill., through the courtesy of Dr. A. E. Meyer.

of immature rats appears to differ from that of sheep pituitary powder in that horse pituitaries seem to produce a greater follicular stimulation as compared to luteinization than those of similar preparations from sheep. This difference is especially noticeable in the case of castrate male horses. Pituitaries from 6 castrate males were prepared in powder form as described above. When this material was injected in saline emulsion in doses varying from 3 to 25 mg. the ovaries of the test rats showed predominantly follicular development, even in cases where the ovaries had increased in size as much as 210 mg. Slight luteinization was noticeable in only 2 cases out of 16 rats so injected. It seems therefore, that the pituitaries of these 6 castrate horses contained primarily follicular stimulating activity with very little luteinizing potentiality. This is in sharp contrast to pituitary powder from sheep which when injected in saline emulsion, in doses sufficient to produce ovaries of similar size, stimulated luteinization in every case.

6610

A Technique for Rendering Tissues Blood-free.

S. W. KLETZIEN, K. W. BUCHWALD AND L. HUDSON.
(Introduced by R. S. Hubbard.)

From the State Institute for the Study of Malignant Disease, Buffalo, N. Y.

The problem of rendering the tissues of a small laboratory animal like the rat, blood-free, must be solved if this animal is used in the study of tissue iron metabolism. In order that a more nearly correct iron content of a tissue may be obtained it will not suffice merely to correct for the iron contained in the blood of such a tissue, nor will the bleeding of the animal through decapitation or through severing a large artery render the tissues blood-free. The solution of this problem, we believe, rests in the development of a perfusion technique whereby the blood may be washed out of the living animal. A method adapted from Whipple's[1] procedure we wish to report here.

The method requires the services of 2 individuals. The equipment includes an ordinary dissecting kit, a ligature needle, a No. 23 hypodermic needle attached by means of a piece of rubber tubing

[1] Whipple, G. H., *Am. J. Physiol.*, 1926, **76**, 693.

to a 50 cc. burette equipped with pinch-cock, an operating board, and ligatures.

The animal is anesthetized with ether and fastened to an operating board, ventral side up. The skin is removed from the neck region extending from the chin, to and beyond the clavicles, and on either side of the median line to the axillae. The external jugular veins are dissected and linen ligatures are placed under the main branches of both veins. The internal carotid arteries are next dissected and one is prepared according to the method of Bethke, *et al.*[2] The latter carotid is, however, not severed to permit the flow of blood until later. The right external jugular vein is now closed to increase the blood flow through the opposite vein by means of an artery clamp. Into the latter vein now supported by ligatures and drawn slightly taut the hypodermic needle is inserted, as far forward anteriorly as possible with the point of the needle towards the heart. A flow of perfusion fluid into the vein assured (Locke's Solution) the needle is fastened in the vein with a ligature. The artery clamp on the opposite vein is removed. The operating board is tilted to an angle of 45° or more, the head of the animal lowermost. While a fine cut is being made in the specially prepared carotid artery, perfusion fluid is allowed to enter the animal. The inflow of the perfusion fluid should compensate as nearly as possible for the outflow of blood in order to maintain an adequate volume of fluid on which the heart may act. After 10 to 11 cc. have entered the animal the branches of the left jugular vein anterior to the point of insertion of the needle are severed to relieve the head of pressure, and after 19 to 20 cc. have entered, the same branches of the opposite vein are severed. These latter operations facilitate the thorough bleeding of the head. After a further introduction of 4 or 5 cc. the remaining intact internal carotid artery is severed and with the introduction of a total of 24 to 25 cc. of perfusion fluid the effluent from the severed carotids is clear and the perfusion is said to be complete.

The various tissues are removed, cut open and placed in saline to remove any superficial blood, and are then allowed to drain on clean cotton gauze and weighed to determine their fresh weight. They are then dried in a constant temperature oven at 100° for 48 hours to determine their moisture content. These tissues after having been ground in porcelain mortars are now ready for chemical analysis.

[2] Bethke, R. M., Steenbock, H., and Nelson, Mariana T., *J. Biol. Chem.*, 1923, **58**, 71.

In the fresh state, following a perfusion as described, the brain and the lungs are milky white in color, the spleen a pinkish red, the liver a deep buff or brown, the kidneys a paler buff color, while the heart is more pale and the muscles have their usual color. The fluid in the dorsal aorta is pinkish in color. The moisture content of these tissues does not indicate that edema has taken place.

Generally speaking, the use of the technique described has made for lower values of iron in tissues. Below are given the different iron contents of the various tissues of rats which have been prepared in different ways. In one instance the animals were killed with ether, in a second the animals were bled from the carotid and in a third according to the new technique. All tissues were removed from the animals in the same manner, placed in saline, and then allowed to drain on clean cotton gauze. The further preparation for analysis was the same in all cases. The iron determination was carried out according to Elvehjem's[3] modification of Kennedy's[4] method. Ashing time and temperature were maintained at a minimum. The samples used in each case were the composites of 11 animals of similar weight, age, sex, and from the same litters. Their average age was 10 weeks and they had been fed the stock ration *ad libitum*. The iron content of this ration averages about 253 mg. per kilo of dry ration.

TABLE I.
Iron Content of Rat Tissues Prepared in Various Ways Expressed on a Dry Weight Basis.

Tissue	Perfused mg. per 100 gm.	Bled mg. per 100 gm.	Not Bled mg. per 100 gm.
Bone	6.95	7.65	8.26
Brain	9.64	10.90	11.50
Heart	32.50	38.00	40.60
Kidney	26.20	26.00	43.50
Liver	60.40	56.60	81.60
Lung	23.00	35.50	64.40
Muscle	10.60	8.75	10.05
Spleen	84.20	93.00	108.50
Testicle	19.00	19.00	19.60

As more refined methods for the determination of iron in small amounts become available we feel that these differences will be amplified.

[3] Elvehjem, C. A., *J. Biol. Chem.*, 1930, **86**, 463.
[4] Kennedy, R. P., *J. Biol. Chem.*, 1927, **74**, 385.

6611

Mineral Metabolism—Copper and Iron.

S. W. KLETZIEN, K. W. BUCHWALD AND L. HUDSON.
(Introduced by R. S. Hubbard.)

From the State Institute for the Study of Malignant Disease, Buffalo, N. Y.

Iron and copper have long been known to play important rôles in respiratory phenomena, oxidation and reduction, enzyme action, pigment formation and in other vital phenomena. The importance of these elements, particularly the former, has been emphasized by the work of Warburg on the respiratory processes occurring in neoplastic tissues. Scott and co-workers[1] have shown that an apparent abnormal iron metabolism obtains in neoplasms. These findings, and those relating to hemochromatosis involvement in primary carcinoma of the liver and the failure in the treatment of neoplasms generally by means of specific chemical agents, have caused us to seek more information as to the metabolism of these elements in the animal body.

The work reported here deals with the influence of large amounts of copper sulphate on the magnitude of the iron assimilation of the various tissues of rats. The copper sulphate was fed as an integral part of an otherwise normal diet. Control animals received the same diet without the addition of copper sulphate. Daily consumption of the respective diets was equalized and recorded. Hence the diets of the 2 groups of animals differed only in the amount of copper as copper sulphate ingested. Distilled water was available to the animals at all times. Three feeding series were run, involving the use of 62 animals, of which 32 served as controls, the remainder receiving the high copper containing diet. All animals were maintained on screens and in individual cages throughout the feeding period of 12 weeks. In each series particular attention was given to securing uniformity of animals in the respective groups, distributing them not only according to weight and age, but also according to sex and litter. The animals were 4 to 5 weeks old when placed on experiment.

The iron intake for all animals averaged 2 mg. per day over the 12-week period. The copper intake of the controls, derived from the natural components of the diet and an inorganic salt mixture, averaged 0.033 mg. per day over the same period. The daily cop-

[1] Scott, G. H., and Horning, E. S., *Am. J. Path.*, 1932, **8**, 329.

per intake of the animals on the high copper diet averaged 5.43 mg. or approximately 165 times that ingested by the controls.

After 12 weeks the tissues of these animals were prepared according to the technique described in the previous paper. Iron determinations were made according to Elvehjem's modification[2] of Kennedy's method.[3] Copper determinations were made according to Elvehjem's and Lindow's modification of the Biazzo method as further modified by Gebhardt and Sommer.[4] Our results follow. In the first 2 series insufficient material prevented our obtaining both copper and iron values on all tissues. With the introduction of the use of chloroform to remove the suspended copper sulphide on precipitation with hydrogen sulphide we are now able to make copper and iron determinations on the same sample of material.

TABLE I.
Iron and Copper Content of Tissues from Rats Receiving a High Intake of Copper as Copper Sulphate (Dry Weight Basis).

Controls					Copper-fed			
No. Animals	Tissue	% H_2O	Fe mg./100 gm.	Cu mg./kilo	No. Animals	% H_2O	Fe mg./100 gm.	Cu mg./kilo
12*	Liver	73.8	68.20	12.40	10*	74.0	82.40	665.00
	Spleen	76.9	†	27.70		77.5	†	20.90
11 (Z)	Liver	75.9	61.00	16.30	11 (Z)	75.0	63.40	735.00
9 (Z)	Bones	21.1	6.15	1.65	9 (Z)	24.9	7.21	1.67
	Brain	77.0	8.90	10.40		76.2	13.10	10.60
	Heart	73.6	34.60	21.20		74.3	50.30	20.70
	Kidney	80.0	22.60	25.60		80.3	24.30	58.50
	Liver	73.2	88.00	10.30		71.0	91.10	301.00
	Lung	76.1	21.60	17.40		78.8	27.30	16.70
	Muscle	72.5	9.55	1.55		71.5	10.50	1.44
	Spleen	74.5	286.00	32.80		74.6	303.00	20.20
	Testicle	86.0	23.40	15.80		84.5	29.40	14.30

* Bled from carotid. (Z) Perfused. † No iron determination made.

As to our findings we wish to point to the small, nevertheless distinct and consistent increases in the iron content of all the tissues analyzed and the definite decrease in the copper content of the spleens of the animals on the high copper diet as compared to the control animals. Whether this signifies an increased utilization of iron by the tissues is not of course established by this limited amount of data. The authors are inclined to believe, however, that the find-

[2] Elvehjem, C. A., *J. Biol. Chem.*, 1930, **86**, 463.

[3] Kennedy, R. P., *J. Biol. Chem.*, 1927, **74**, 385.

[4] Gebhardt, H. T., and Sommer, H. H., *Ind. and Eng. Chem., Anal. Ed.*, 1931, **3**, 24.

ings do so signify. If iron is better utilized by the tissues in the presence of copper then we may ascribe to copper an additional function in iron metabolism other than its function in making iron available for hemoglobin synthesis. We offer no explanation for the decrease in the copper content of the spleens of animals receiving the high copper diet except that they seem unusual. Pending further experiments involving the use of higher levels of iron intake and more varied intakes of copper we reserve the privilege of making a more definite interpretation of the results here reported.

6612

Production of Exclusively Thecal Luteinization and Continuous Oestrus with Anterior-Pituitary-Like Hormone.

H. SELYE AND J. B. COLLIP.

From the Department of Biochemistry, McGill University, Montreal, Canada.

The fact that rats do not respond to anterior-pituitary-like hormone (A.P.L.) in the first days of life has been observed repeatedly, and the conclusion has been drawn that they should not be used as test objects for this hormone earlier than the 18th to the 21st day of life. In an attempt to find an explanation for this fact we injected a series of 30 rats daily with A.P.L., starting on the 6th day of life. Although no mature follicles or corpora lutea had been formed after 10 injections and the ovary did not differ macroscopically from that of an untreated rat of the same age, histological examination of this organ reveals very pronounced changes. The thecal cells were very much enlarged and assumed the appearance of corpus luteum cells, whereas the granulosa cells were not luteinized and no signs of ripening of the follicles could be detected. These experiments show that at a very early age A.P.L. is unable to induce follicular maturation or the formation of normal or atretic corpora lutea; however, it does lead to luteinization of the thecal cells and thereby to the formation of thecal corpora lutea. These structures are not very prominent and therefore they can be readily overlooked upon macroscopical examination. (Fig. 1.) Histologically they are composed of a peripheral ring of corpus luteum cells and a central part of a few rows of normal granulosa cells in the center of which the ovum is included. (Fig. 2.)

It is difficult to find a satisfactory explanation for these observa-

FIG. 1.
To the left, thecal corpora lutea in the ovary of a rat treated with A.P.L. from the 6th to the 21st day of life. To the right, real corpora lutea and one blood point in the ovary of a rat treated with A.P.L. between the 21st and 26th day of life.

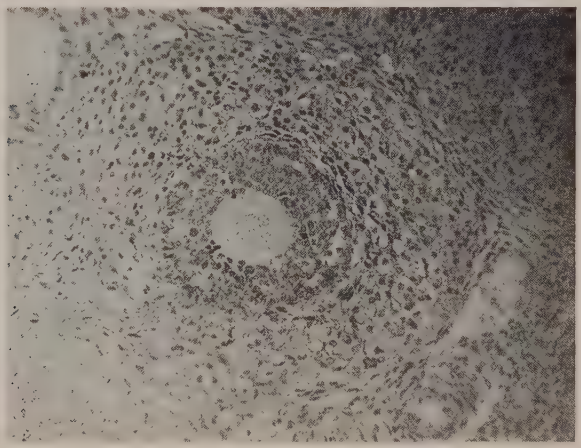


FIG. 2.
High magnification of thecal corpus luteum in the ovary of a rat treated with A.P.L. from the 6th to the 16th day of life. Luteinized thecal cells in the periphery. Normal granulosa cells and ovum in the center. No signs of follicular maturation.

tions. It is remarkable, however, that the hypophysectomized rat shows a very similar ovarian reaction when treated with A.P.L.[1] In

[1] Collip, J. B., Selye, H., and Thomson, D. L., *Nature*, 1933, **131**, 56.

the absence of the pituitary we could never induce maturation of the follicles or luteinization of the granulosa cells with A.P.L., but the luteinization of the theca cells is very conspicuous. This might suggest that the stimulation of granulosa elements is only possible in the presence of a pituitary factor which would obviously be lacking in the hypophysectomized animal and which, perhaps, cannot be supplied adequately by the immature pituitary during the first days of life.

If a thecal luteinization has been produced during the first days of life and the administration of A.P.L. is continued to the 26th day, the normal reaction of the ovary fails to develop. Whereas the control litter-mate receiving A.P.L. between the 21st and 26th day of life only shows maturation of follicles and corpus luteum formation, the ovary of the rat receiving the same amount of this hormone from the 6th until the 26th day shows nothing but thecal luteinization. Whether this phenomeon is due to an inhibition of follicular maturation by the corpus luteum hormone formed in the thecal cells is meanwhile open to discussion.

Following the formation of these thecal corpora lutea continuous oestrus has been observed both in hypophysectomized rats and in the animals of the present experimental series. As no signs of maturation could be detected in the granulosa, we have to assume that the luteinized thecal cells are responsible for the oestrus.

6613

Further Studies on the Exophthalmos in Rabbits Produced by Methyl Cyanide.*

DAVID MARINE, S. H. ROSEN† AND ANNA CIPRA.

From the Laboratory Division, Montefiore Hospital, New York.

Chronic, progressive, bilateral exophthalmos has been produced in more than 150 prepubertal rabbits maintained on a diet of alfalfa hay and oats by the daily intramuscular injection of 0.05-0.1 cc. of methyl cyanide. [1, 2] Feeding fresh vegetables markedly inhibits its

* Aided by a grant from the Ella Sachs Plotz Foundation.

† Fellow of the Montefiore Hospital.

[1] Marine, D., Spence, A. W., Cipra, A., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 822.

[2] Marine, D., Baumann, E. J., *Trans. Assn. Am. Phys.*, 1932, **47**, 261.

development. Exophthalmos may appear as early as the 14th day and as late as the 60th day after beginning the cyanide injections. Males are much more susceptible and some breeds (Dutch) are more susceptible than others (Belgian). The degree of exophthalmos obtained has been highly variable in rabbits of the same age, sex and breed, but it is always proportional to the degree of thyroid hyperplasia (goiter) present.

We believe it is established that the initial cause of the exophthalmos is dependent primarily upon an increased tonicity and spastic contraction of the smooth muscles of the orbit and eyelids which are brought about by stimulation through the autonomic nervous system.

1. Dividing the cervical sympathetic trunk in 3 rabbits below the superior cervical ganglion definitely diminished the exophthalmos on the operated side when the animals were quiet, and particularly reduced the spasm of the lower eyelid. If the animal was disturbed, however, the exophthalmos became as marked as on the intact side.

2. Removal of the superior cervical ganglion permanently abolished the exophthalmos on that side in 6 animals.

3. Curetting the medulla of both suprarenals was without effect on existing exophthalmos in 3 animals, nor did it hasten or delay the onset of exophthalmos. Curetting the right suprarenal medulla and removing the left suprarenal gland in 18 rabbits was also without definite effect.

4. Thyroidectomy in 13 prepubertal rabbits hastened the onset of exophthalmos and increased it when performed after exophthalmos had developed. Thyroidectomy decreased the percentage of prepubertal rabbits resistant to the development of exophthalmos from 20-30% to 0.

Since exophthalmos develops in rabbits with intact thyroids only when they develop marked thyroid hyperplasia (goiter), one may conclude that protection against cyanide exophthalmos is in part dependent upon the presence of the iodine containg hormone of the thyroid. So far, we have not been able to abolish the exophthalmos in thyroidectomized rabbits by administering desiccated thyroid. (In several animals this drug has caused a temporary increase.) The administration of iodine to rabbits with intact thyroids, however, prevents its occurrence, as does the administration of fresh plant and fruit juice concentrates (ascorbic acid). The exophthalmos, therefore, depends in part upon thyroid insufficiency. Gley[3] noted

[3] Gley, E., *C. R. Soc. Biol.*, 1910, **68**, 858.

the spontaneous occurrence of exophthalmos in 2 thyroidectomized young rabbits. The exophthalmos of Graves' disease also appears to depend in part upon a relative thyroid insufficiency, since it may develop after and is frequently made worse by partial thyroidectomy and since the most beneficial treatment[4, 5, 6] has been with desiccated thyroid or thyroxin and iodine.

6614

Measurement of the Circulation Time with Saccharin

ARTHUR M. FISHBERG, WILLIAM M. HITZIG AND FREDERICK H. KING.
(Introduced by E. H. Fishberg.)

From the Medical and Laboratory Divisions, Mount Sinai Hospital.

Among the methods used for the measurement of the circulation time in man are those in which a foreign substance is injected into a vein and the time of its arrival in the capillaries of the tongue is signalled by a sensation of taste. Winternitz, Deutsch, and Bruell[1] have used the interval between the intravenous injection of sodium dehydrocholate and the appearance of a bitter taste as a measure of the circulation time. Both they and Tarr, Oppenheimer, and Sager (personal communication) have obtained excellent results in the clinical application of the method.

We have found that soluble saccharin (sodium benzosulphinid) is admirably adapted to the estimation of the circulation time between the antecubital veins and the capillaries of the tongue. Soluble saccharine possesses the following advantages: 1. It stimulates the taste buds in very high dilution. 2. It is very soluble, so that only a small volume of solution is needed. This is important because the injection can be performed rapidly and the saccharin is contained in a small blood volume, with resultant sharp definition of both the time of injection and of arrival in the tongue. 3. It is apparently entirely harmless in the quantities used. In over 100 individuals no unpleasant reactions were encountered. Paravenous infiltration causes no necrosis. In several instances the circulation time was measured twice, and once even 3 times within a few min-

[4] Zimmerman, L. M., *Am. J. Med. Sci.*, 1929, **178**, 92.
[5] Ruedemann, A. D., *J. Am. Med. Assn.*, 1931, **97**, 1700.
[6] Benedict, W. L., *Arch. Ophth.*, 1933, **9**, 1.
[1] Winternitz, Deutsch, and Bruell, *Med. Klin.*, 1931, **27**, 986.

utes, but no reactions were observed. 4. The measurement can be repeated as soon as desired. The residual saccharin in the blood does not interfere with perception of the newly injected substance.

The measurement is performed as follows: 2.5 gm. of soluble saccharin is dissolved in 2 cc. of distilled water by heating. The subject reclines in a comfortable position and is told to call out when he experiences a sweet taste. The solution is injected rapidly into a large antecubital vein (less than 0.5 seconds is required). The time that elapses until perception of the sweet taste is measured. The subject usually describes the sweet taste as passing with great rapidity from the base to the tip of the tongue and quickly disappearing. He should be instructed to relax and not to hold his breath following the insertion of the needle, for this slows the venous return to the heart.

One hundred individuals, mostly hospital patients, suffering from a variety of complaints, were tested. In only one of these was the endpoint not definite enough for a reading. In 63 subjects there was no reason to suspect abnormality of circulation. The circulation times in these 63 "normals" were as follows:

Circulation Time sec.	No. Individuals
9- 9¾	8
10-10¾	18
11-11¾	6
12-12¾	11
13-13¾	12
14-14¾	3
15-15¾	5

Repeated injections in the same subject check closely.

In circulatory failure, as found by previous investigators, the circulation time is markedly protracted, in severe cases to over 40 seconds. The circulation time is undoubtedly a very valuable index of circulatory failure. In fact, the slowing of the circulation time in circulatory insufficiency appears to be proportionately greater than the diminution in the cardiac output as revealed by recent investigations with the acetylene method.

6615

Neurotropism of Vesicular Stomatitis Virus.

HERALD R. COX AND PETER K. OLITSKY.

From the Laboratories of the Rockefeller Institute for Medical Research, New York.

The virus of vesicular stomatitis is peculiarly epitheliotropic (dermotropic) in the guinea pig: only the pad tissue of the dermal surface is uniformly susceptible.[1, 2, 3]

Through the kindness of Dr. W. E. Cotton, samples were sent us of Indiana and New Jersey strains of virus propagated in guinea pig pads for several years. After 20 consecutive transmissions of the experimental disease in pads, both strains were filtered through Seitz' discs and were inoculated intracerebrally in respective guinea pigs.* The amount injected was 0.15 cc. of filtrate, derived from 1:10 suspensions of affected pad tissue ground in hormone broth at pH 7.5. Both the New Jersey and the Indiana materials produced a striking reaction.

From 2 to 5 days after injection, the animals exhibited first weakness, then paresis of both posterior extremities, which became progressively more marked and reached, within 2 to 3 days, the stage of complete flaccid paralysis. No other signs of disturbance, including fever, were detectable. About 60% of the animals died during the paralytic stage. The survivors either recovered partially, showing merely an ataxic gait, or were left with paralyzed legs. To the present, 6 brain to brain passages have been made in the guinea pigs. Practically all of the animals proved susceptible to intracerebral inoculations and, after several such passages, the action of the virus became regular.

Microscopic study of the organs removed at the height of reaction revealed changes only in the cerebrospinal system. The meninges and choroid plexus showed only invasion by few mononuclear cells. The brain itself exhibited general edema, slight perivascular mononuclear infiltration, and inconspicuous, diffuse, monocytic reaction, together with mild increase of glia nuclei. Many ganglion cells revealed various stages of degeneration and occasional neurono-

[1] Olitsky, P. K., *J. Exp. Med.*, 1927, **45**, 969; Olitsky, P. K., Traum, J., and Schoening, H. W., *Technical Bull.*, No. 76, U. S. Dept. of Agriculture, 1928, 172.

[2] Hoffman, D. C., *J. Exp. Med.*, 1931, **53**, 43.

[3] Wagener, K., *J. Am. Vet. Med. Assn.*, 1932, **80** (N. S. **33**), 39.

* All operative procedures were carried on under ether anesthesia.

phagia. The spinal cord was the seat of corresponding changes; the membranes, except for slight mononuclear cellular infiltration, were normal. The cord itself was edematous and the nerve cells were degenerated. The nuclei of many of the nerve cells contained an acidophilic, granular material. Inclusion bodies were not detected.

The identification of the neurotropic nature of the vesicular stomatitis virus is based first on the characteristic local lesions following, invariably, the injection of pads with active brain tissue. These reactions are more marked than those obtained with ordinary pad virus, thus indicating a greater activity of the neurotropic virus. The second means of identification depends on cross-immunity, that is, animals recovered from a brain inoculum are immune to the original vesicular stomatitis virus injected in their pads, and conversely, guinea pigs recovered from pad inoculation of original virus develop resistance in their pads and in their central nervous system against active cerebral tissue.

Brain material retained its neurotropism after storage for at least 32 days in 50% glycerol. It was also found to be active in the cerebrospinal system of rabbits and white mice. Experiments with mice are still in progress.

In conclusion, a neurotropic strain of vesicular stomatitis virus has been described, which may prove of value when bacteria-free, yet unfiltered, active material is desired—heretofore unobtainable with pad virus.

6616

Further Studies on Neurotropism of Vesicular Stomatitis Virus.

HERALD R. COX AND PETER K. OLITSKY.

From the Laboratories of The Rockefeller Institute for Medical Research, New York.

In a preceding report[1] we have shown that intracerebral inoculation of guinea pigs with the virus of vesicular stomatitis of horses induces characteristic degenerative lesions in the organs of the central nervous system, and after the virus had become fixed by

[1] COX, H. R., and Olitsky, P. K., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 653.

several brain passages in guinea pigs, it exhibited neurotropic action in white mice and rabbits.

After 25 consecutive transmissions of the virus in guinea pig pads, the Indiana and New Jersey strains, already described,[1] were filtered through Seitz' discs and inoculated intracerebrally into white mice.* The amount injected was 0.03 cc. of filtrate, derived from 1:10 suspension of affected pad tissue ground in hormone broth at pH 7.5.

Within 30 to 40 hours after injection of either the Indiana or New Jersey strain, the mice develop marked hypersensitiveness, ruffling of the hair, tremors, weakness of the legs, ataxia, and spastic paralysis of the posterior extremities associated with generalized involuntary muscular contractions. The disease is uniformly fatal 48 to 72 hours after inoculation. To the present, 8 brain to brain passages have been made, and all the mice inoculated have developed symptoms.

The histopathological changes in the brain consist of edema and small, localized hemorrhages in the cerebrum. The meninges, however, are not involved. The characteristic lesion is the pronounced necrosis of most of the neurones, especially those of the motor nuclei in the brain stem. The cerebellum is also affected and a massive destruction of the Purkinje cells occurs along with invasion of their layers by an occasional monocyte. The spinal cord reveals a similar necrosis of the neurones.

The identification of the virus as that of vesicular stomatitis is based on cross immunity tests in the guinea pigs, using the guinea pig pad and mouse brain viruses.

White and hooded rats were also found to react to the intracerebral inoculation of filtered suspensions of guinea pig pads showing the vesicular stomatitis lesions. Rabbits, on the other hand, proved to be completely resistant, as previously confirmed.[2]

In conclusion, the neurotropism of vesicular stomatitis virus has again been demonstrated and the white mouse shown to be suitable as a substitute for the guinea pig as an experimental animal.

In view of the fact that vesicular stomatitis virus may be regarded as having a generic relationship to the incitant of foot-and-mouth disease,[3] the use of the white mouse may prove advantageous in experimental work with the latter virus.

* Ether anesthesia was used in all operations on animals.

[2] Olitsky, P. K., *J. Exp. Med.*, 1927, **45**, 969.

[3] Olitsky, P. K., in Rivers, T. M., Filterable Viruses, Williams and Wilkins Company, Baltimore, 1928, 205.

6617

Infection in Mice Following Nasal Instillation of Louping-Ill Virus.

L. T. WEBSTER AND G. L. FITE.

From the Laboratories of the Rockefeller Institute for Medical Research, New York.

Regular transmission to mice of a fatal infection following intranasal instillation of louping-ill virus, apparently not accomplished until now,[1, 2, 3, 4, 5] has been effected by the employment of brother-sister inbred strains of mice.[6]

White-face and black-and-tan mice, brother to sister inbred, for at least 10 generations and maintained on a standard diet and uniform routine in a special breeding room, have reacted in a consistent and characteristic manner to the virus obtained from T. M. Rivers. This virus, the Moredun Strain from Mackie, has proved infective for mice and monkeys when injected intracerebrally and gives rise to the characteristic signs and histopathology of louping-ill.[2, 3, 4, 5]

Intranasal transmission has been carried out as follows: Brains from 2 to 4 mice dead of the experimental infection induced by intranasal or intracerebral injection are removed aseptically, cultured, weighed, ground in a sterile mortar, diluted in normal saline, and again cultured for contaminants. Duplicate batches of 20 mice each are given 0.02 cc. of the diluted virus into the nares through a 0.25 cc. tuberculin syringe and blunt needle. Care is taken not to touch or injure the nasal tissues. The mice are then placed in individual jars and observed over a 4 week period. Animals found dead are autopsied and cultured for bacterial growth. Passage in series from the brains of mice dying from intranasal instillation to the nasal passages of normal mice has been continued in parallel series successfully and uniformly 8 times.

When white-face mice are used dilutions of virus from 1 to 2 to 1 to 20 result in an incubation period of 6 to 7 days, followed by rapid and progressive development of hyperesthesia, tremors, inco-

[1] Pool, W. A., Brownlee, A., and Wilson, D. R., *J. Comp. Path. and Therap.*, 1930, **43**, 253.

[2] Greig, J. R., Brownlee, A., Wilson, D. R., and Gordon, W. S., *Vet. Rec.*, 1931, **11**, 325.

[3] Alston, J. M., and Gibson, H. J., *Brit. J. Exp. Path.*, 1931, **12**, 82.

[4] Hurst, E. W., *J. Comp. Path. and Therap.*, 1931, **44**, 231.

[5] Brownlee, A., and Wilson, D. R., *J. Comp. Path. and Therap.*, 1932, **45**, 67.

[6] Webster, Leslie T., *J. Exp. Med.*, 1933, in press.

ordination, partial paralysis, especially of the hind limbs, prostration, and finally death between the eighth and tenth days in practically every case. When higher dilutions of the virus are given, or when black-and-tan mice are tested, the incubation period and duration of signs of disease and of life lengthen and the percentage mortality falls below 100%.

The most conspicuous and consistent anatomical changes associated with the intranasal infection appear to be necrosis of pyramidal cells in the lobus piriformis and cornu ammonis of the cerebrum and acidophilic intranuclear bodies in certain glial cells throughout the brain and cord and in cells of the choroid plexus in preparations fixed with Zenker-acetic and colored with Giemsa stain. The necrotic cerebral lesion has been mentioned but not stressed by Hurst[4]; the intranuclear bodies observed jointly by Rivers and Webster in this material have not been referred to previously. Besides these characteristic changes, necrosis of Purkinje cells, motor neurons in pons, medulla, and cord and infiltration of mononuclear cells around blood vessels and dying neurons are found in a percentage of cases. These lesions have been reported by others.[4, 5]

The virus does not affect rabbits when inoculated intracerebrally. The necroses and intranuclear bodies do not appear in preparations of normal mice. The virus does not seem, therefore, to be contaminated with herpes or unknown mouse virus.

6618

Weight of Pituitary and Thyroid of the Rat at Various Stages of the Oestrus Cycle.

DOROTHY H. ANDERSEN. (Introduced by A. M. Pappenheimer.)

From the Department of Pathology, College of Physicians and Surgeons, Columbia University.

In the recent literature on the relation of the pituitary gland to reproduction there have been several notes concerning changes in the histological picture or in the physiological activity of the pituitary associated with changes in the oestrus cycle. Charipper and Haterius[1] observed an increase in the size and number of the basophiles during oestrus, although the cells were not counted or meas-

[1]Charipper, H. A., and Haterius, H. O., *Anat. Rec.*, 1932, **54**, 15.

ured. Wolfe and Cleveland[2] found an increase in the eosinophilic cells during oestrus and an increase in granules in the basophilic cells during late dioestrus. Smith and Engle[3] found an increase in the gonad-stimulating hormone of the anterior pituitary during dioestrus as tested by the implant method. Wolfe[4] confirmed this.

The observations presented here were made on mature normal

TABLE I.

Weight of Pituitary and Thyroid of the Rat at Various Stages of Oestrus Cycle.

Stage of Cycle	No.	Age Days	Body Wt. gm.	Pituitary mg.	Pituitary mg./kg.	No.	Age Days	Body Wt. gm.	Thyroid mg.	Thyroid mg./kg.
Pro-oestrus	5	110	165	10.0±0.8	59.0±1.6	5	110	165	15.6±1.5	94.0±10.0
Oestrus	23	128	188	12.3±1.3	65.1±1.9	34	119	177	19.0±1.6	114.0±13.0
24 hours after oestrus	0	—	—	—	—	9	102	150	15.8±2.2	106.0±24.0
48 hours after oestrus	14	129	179	10.5±1.0	58.9±2.2	17	123	171	18.8±3.6	112.0±12.0
Over 48 hours after oestrus	8	102	170	9.1±0.9	53.6±4.3	7	102	170	18.2±1.6	107.0±4.5
Day before expected oestrus	3	87	153	7.7±0.9	50.6±2.9	3	87	153	16.4±2.0	107.0±23.0

Figures for age and weight represent the mean for the group.
Mg./kg. refers to mg. of fresh pituitary per kg. body weight.

[2] Wolfe, J. M., and Cleveland, R., Abstract by Wistar Institute, in press.
[3] Smith, P. E., and Engle, E. T., *Anat. Rec.*, 1929, **42**, 38.
[4] Wolfe, J. M., *Am. J. Anat.*, 1931, **48**, 391.

female rats ranging from 3 to 6 months in age. Infected animals and animals having irregular oestrus cycles or cycles over 6 days in length were excluded.

Animals of the same strain were kept under the conditions and on the diet described previously.[5, 6] Vaginal smears were made at intervals of 12 hours or, in most cases, of 8 hours, and oestrus was defined as the period at which an abundance of cornified cells was obtained, after the preceding smear had shown nucleated epithelial cells. Dioestrus was defined as 48 or more hours after this time. Three animals with very regular cycles were killed 20 hours before the expected onset of the next oestrus. The animals were killed with chloroform and the pituitary, thyroid and adrenal were quickly removed and weighed in a closed weighing bottle to 0.1 mg. The data on the adrenal have been previously reported.[6] The pituitary weights are thought to be accurate to about 0.2 mg. since the gland need not be dissected from surrounding tissue. The thyroid weights are much less so because of the difficulty of accurate and rapid dissection. They also include the parathyroids.

The mean actual and relative weight of the pituitary and the probable error of this mean were calculated for each group of animals. The resulting figures indicate definite and significant increase in the weight of the pituitary during oestrus, with gradual and progressive decrease during dioestrus. This decrease in animals killed 52-72 hours after oestrus amounted to approximately 20% of the relative weight at dioestrus.

The thyroid showed no significant change in weight, but the wide variation in thyroid weight in the entire series is sufficient to obscure any except an extreme change.

6619

Cultivation of Mycobacterium Leprae.

EARL B. MCKINLEY AND ELIZABETH VERDER.

From the Department of Bacteriology, Hygiene and Preventive Medicine, School of Medicine, George Washington University, Washington, D. C.

McKinley and Soule[1] reported the successful cultivation of *Mycobacterium leprae*, obtained from Puerto Rican lepers, on several

[5] Andersen, D. H., *J. Physiol.*, 1932, **74**, 49.

[6] Andersen, D. H., and Kennedy, Helen S., *J. Physiol.*, 1932, **74**, 247.

[1] McKinley, Earl B., and Soule, Malcolm H., *J. Am. Med. Assn.*, 1932, **98**, 361.

culture media. Subsequent reports were made by Soule and McKinley[2, 3] when their nonchromogenic strain of acid-fast bacilli, believed to be the true *Mycobacterium leprae,* had been carried through the eighth and sixteenth generations respectively, the latter representing cultivation over a period of 18 months. Experimental protocols were also presented dealing with suggestive experimental lesions produced in 2 species of monkeys. In the cultivation work it was apparent that the leprosy microbe was maintained on artificial media with greater difficulty with each generation or transfer. In the sixteenth generation, so-called, after the organism had been on artificial media for some 18 months, only 2 definitely positive cultures resulted. One of these cultures has been employed in an attempt to ascertain better methods of cultivation.

A logical procedure was to attempt cultivation in embryonic tissue. Minced chick embryo 7 to 11 days old, was washed and suspended in Tyrode's solution. Human embryonic tissue has also been employed, but with less success. In the chick embryo, suspension, growth of the original *Mycobacterium leprae* has been stimulated. Growth is obtained within 5 days under CO_2 and O tension as well as under ordinary atmospheric conditions in the incubator. Several additional generations or transfers have been added to the 16 previously reported with this organism. Growth of *Mycobacterium leprae* has also been obtained with human embryonic spleen tissue suspended in Tyrode's solution, but this tissue is more difficult to obtain and is no longer employed in our routine culture work. With the young chick embryo tissue medium we are now able to cultivate, apparently indefinitely, this strain of *Mycobacterium leprae.*

There has always been doubt, when claims have been made for the cultivation of the true causative agent of leprosy, that actual cultivation *in fact* has been accomplished. None of the methods seemed certain and many different varieties of organisms have been isolated from human leprosy materials. The organism isolated from lepers by one of us with Soule, however, has differed remarkably in its habits and the conditions necessary for growth and multiplication. Furthermore, suggestive lesions have been obtained with it in experimental animals and the organism, with all the difficulties encountered in maintaining it on artificial media, has remained viable for nearly 2 years, surviving *only* under the special gaseous

[2] Soule, Malcolm H., and McKinley, Earl B., *Am. J. Trop. Med.*, 1932, **12**, 1.
[3] Soule, Malcolm H., and McKinley, Earl B., *Am. J. Trop. Med.*, 1932, **12**, 441.

conditions described. In view of these facts the work indeed has seemed very encouraging.

Recently we obtained leprosy nodules through the kindness of Dr. O. E. Denney at Carville, La., and with the chick embryo tissue method have attempted to isolate Hansen's bacillus from these fresh cases. If our original organism was the authentic leprosy bacillus it should be possible to cultivate, in tissue medium, the true leprosy germ from fresh lepromata. We had nodules from 3 different cases, emulsions of which were all contaminated with non-acid-fast organisms when received. Such emulsions also contained a vast number of acid-fast organisms, presumably Hansen's bacillus. These emulsions we have treated and concentrated with 3% sodium hydroxide to destroy contaminants and have succeeded in cultivating the acid-fast organism from each of these 3 cases in young chick embryo tissue suspended in Tyrode's solution as we have the older Puerto Rican strain. Isolation and growth of acid-fasts from fresh human leprosy tissue seems to be as easily accomplished as the continued growth of our older strain. We believe this presents new and convincing evidence that in these cultivation studies we have without doubt been dealing with the actual causative agent of leprosy, if Hansen's bacillus is to be accepted as the cause of this disease. These strains of acid-fasts which we are able to cultivate from leprosy lesions do not grow on any artificial mediums, in so far as we have tested the several ordinary laboratory media, under ordinary atmospheric conditions. Only in the tissue medium does actual multiplication take place under ordinary atmospheric conditions and it is presumed that in such tissue media we have a CO_2 tension similar to that obtained under the artificial conditions employed by us, as well as other elements which favor multiplication of the microbe under study.

6620

Egg-Oyster Media for the Cultivation of Acid-Fast Bacteria.

EARL B. MCKINLEY and ELIZABETH VERDER.

From the Department of Bacteriology, Hygiene and Preventive Medicine, School of Medicine, George Washington University, Washington, D. C.

Since oysters have been shown to be a good source of vitamins A, B, C, and D as well as to contain in considerable amounts the inorganic elements iron, copper, manganese, zinc, lead, arsenic and

iodine,[1] the possibility of the use of oysters in culture mediums was suggested. It was found that many organisms, including the group of acid-fast bacteria, grow quite luxuriantly on certain of the mediums prepared. Two solid egg-oyster mediums, one made with the yolks and the other with the whites, and a hormone oyster infusion broth have proven to be the most satisfactory of all preparations.

Six strains of *Mycobacterium tuberculosis* (H 37, R_1, a virulent human strain obtained from Dr. Novy, a virulent bovine strain, a virulent avian strain and an avirulent strain) and 9 strains of chromogenic acid-fast bacteria isolated from lepers by several investigators all give typical growth on both the egg white and egg yolk oyster mediums. The strain of *M. leprae* isolated by McKinley and Soule[2, 3, 4] has been successfully cultivated on the egg white oyster when incubated in an atmosphere of 10% carbon dioxide and 40% oxygen for 30 days, but does not appear to multiply on the egg yolk oyster medium when incubated under similar conditions. The value of these mediums for the isolation of acid-fast organisms is being studied further.

The egg oyster mediums are prepared by mixing (a) 2 parts of egg white with 1½ parts of minced oyster; or (b) 1½ parts of egg yolk, ½ part of whole egg and 2 parts of minced oyster. The pH of each mixture is adjusted to about 8.2. The tubes are placed in an inspissator at a temperature of 70 to 80°C. immediately upon filling so that the tissue does not settle to the bottom of the tubes before coagulation. After coagulation the temperature is brought to between 80 and 90°C. and sterilization is carried out at this temperature for one hour on each of 4 successive days. The oyster hormone infusion broth is made as Huntoon's[5] hormone medium broth with the substitution of an equal amount of minced oyster for the ground beef heart and the omission of the laked blood.

[1] Levine, H., Remington, R. E., and Culp, F. B., *J. Nutrition*, 1931, **4**, 469.

[2] McKinley, Earl B., and Soule, Malcolm H., *J. Am. Med. Assn.*, 1932, **98**, 361.

[3] Soule, Malcolm H., and McKinley, Earl B., *Am. J. Trop. Med.*, 1932, **12**, 1.

[4] Soule, Malcolm H., and McKinley, Earl B., *Am. J. Trop. Med.*, 1932, **12**, 441.

[5] Levine, M., and Schoenlein, H. W., A Compilation of Culture Media, Williams and Wilkins, Baltimore, 1930.

6621

Studies on a Medium Yielding the Filtrable Phase of the Tubercle Bacillus.

RALPH R. MELLON AND L. W. FISHER.

From the Western Pennsylvania Hospital, Institute of Pathology, Pittsburgh.

In recent studies[1, 2, 3] on a filtrable phase in a life cycle of the avian tubercle bacillus we have stated that a "modified" Kendall medium was employed, but that further study of it was under way in order to assess more clearly the environmental factors responsible for the phenomenon. This represents a progress report on that work.

Technique. Human brain is ground and extracted 3 times with 4 volumes of 95% alcohol, then once with 4 volumes of benzol. Extractions are conducted at 37°, with 2 days for each one with occasional stirring. The residue should be waxy in character, instead of fibrous as is the case for intestine. If this does not obtain, we cut the time of benzol extraction to ½, ⅓, or more. White, powdery residues have not proved as efficacious as amber, waxy ones. Two percent of the dried residue has been used with Tyrode's solution prepared according to the footnote in Kendall's paper,[4] but with the exception that sodium bicarbonate and glucose are omitted. After autoclaving, the reaction is not adjusted with sodium bicarbonate or any other alkali.

There were 2 reasons for employing brain. First, because the strains of tubercle bacilli (Avian S and Bovine III) with which we worked refused to grow in the intestinal K medium; and second, because we wished to take advantage of the possible nutritious influence of the lipoids whose cultural bearing we suspected from previous unpublished experience with this organism. Then the curious partition coefficients of some of the brain lipoids, the phosphatids, facilitated their retention in the dried residue. A suggestive indication of the lipoid function is the fact that if we extract all of them growth is doubtful, with the production of granules and dissociation changes negative. Moreover, when certain of these extracted

[1] Mellon, R. R., and Fisher, L. W., *J. Bact.*, 1932, **23**, 18.

[2] Mellon, R. R., and Fisher, L. W., *J. Inf. Dis.*, 1932, **51**, 117.

[3] Mellon, R. R., Fisher, L. W., and Richardson, Ruth D., PROC. SOC. EXP. BIOL. AND MED., 1932, **30**, 80.

[4] Kendall, A. I., *Northwestern Univ. Bull.*, 1931, **32**, 8, 1.

lipoids are replaced in the medium, a definite affinity for them on the part of the growing organism has taken place.

But a second factor, apparently significant in its importance, is a reducing substance present in somewhat variable amounts in different brains, indicated by the partial reduction of methylene blue when the medium is let stand at 30° in the incubator for 3 to 5 days. There is thus effected a very definite change in its oxidation-reduction potential. The importance of this reducing substance is suggested by the fact that a human brain which contained no trace of it has consistently refused to grow the tubercle bacillus, even though it showed 5% of lipoids on extraction. A sheep's brain, containing a very small amount of the substance and whose lipoid content is yet undetermined, shows a small amount of growth; while a human brain containing enough to effect approximately a 50% reduction of methylene blue shows an excellent growth of the organism—avian tubercle bacilli, S form—when grown for 2 or 3 weeks at 30°C. This is the temperature at which most of the experiments were conducted. Present data, therefore, point to the rôle of 2 factors at least. Growth occurs in this medium diffusely or as a downy precipitate.

When peptone broth is employed it merely replaces the Tyrode's solution. A few such experiments were run with as high as 3% peptone for the purpose of testing Kendall's theory regarding its antagonistic effect in this type of dissociation. Such antagonistic effect was not observed. This, together with the negative results with the perfectly fat-free residue, leads one to suspect that the mechanism of growth and dissociation is connected with one or more of the chemical factors which we still have under investigation, rather than with unaltered(?) protein as Kendall believes.

Not only with intestine have we failed to get positive filtrates, but almost wholly with lung and liver K media. The relative constancy (25-30%) with which we succeeded with brain medium would seem promisingly to limit the mechanism to some chemical factors fairly specific perhaps, for brain. By the same token, no support is afforded a contamination hypothesis in explanation of the origin of these non-acid-fast forms. Moreover, their dissociation and isolation occur at times in the original, or the reinoculated, media[2] *without filtration;* and certain of such isolated cultures in their early generations are filterable through Berkefeld N candles that have never been in contact with K media. In other words, they conform to Hadley's G-types. Such stabilized non-acid-fast cultures (cocci and diphtheroids) have been transformed to typical

acid-fast strains of an extreme and rather unique R form of the avian bacillus as previously reported. More recently this R form has been dissociated to an avian S culture, serologically and in virulence essentially the same as the original. Thus the last step in the cycle is complete.

Summary. Human brain from which the lipoids have been partially extracted with alcohol and benzol has, in our hands, been successful in yielding the filtrable and non-acid-fast cyclostages of the avian S tubercle bacillus. This appears to be due to two chief factors: (1) the lipoids functioning possibly in a metabolic way; (2) a reducing substance which provides an oxidation-reduction potential of suitable range.

6622

Replacement of Gonadotropic Action of Pituitary in the Hypophysectomized Rat.

J. B. COLLIP, H. SELYE, D. L. THOMSON AND J. E. WILLIAMSON.

From the Department of Biochemistry, McGill University, Montreal.

We have pointed out that the administration of anterior-pituitary-like hormone (A.P.L.) to the hypophysectomized rat cannot prevent the degenerative changes in the ovary which invariably occur after the removal of the pituitary.[1] We also found that it is impossible to induce maturation of follicles and corpus luteum formation in the hypophysectomized rat with A.P.L. injections. In the absence of the hypophysis, A.P.L. leads only to the luteinization of thecal cells but it does not act upon the ovary as it would under normal conditions. The present experiments show us that it is possible to obtain both follicle maturation and corpus luteum formation in the hypophysectomized rat if certain pituitary extracts are administered simultaneously with A.P.L.

In the first series, 6 rats were hypophysectomized when 22 days old, and from that time they received 0.5 cc. of a 0.5% aqueous ammonia extract (1 cc. = 1/10 gm. of anterior lobe tissue), and 25-50 units of A.P.L. daily for 9 days. They all showed squamous vaginal smears on the fourth to the sixth day, and the histological appearance of their ovaries was the same as that of normal immature

[1] Collip, J. B., Selye, H., and Thomson, D. L., *Nature*, 1933, **131**, 56.

females receiving A.P.L. Numerous corpora lutea and mature follicles had been formed in these immature rats in the absence of living pituitary tissue.

In the second series, 7 postpubertal female rats, weighing 72-129 gm., were hypophysectomized and treated with 100 units of A.P.L and ¼ cc. of the same ammoniacal pituitary extract daily for 14 to 24 days. At autopsy they showed numerous corpora lutea, some of them quite recent (Fig. 1). Their ovaries were enlarged, weighing between 43 and 156 mg. Similar results were also obtained with the combination of A.P.L. and aqueous acetic acid extract of the pituitary.

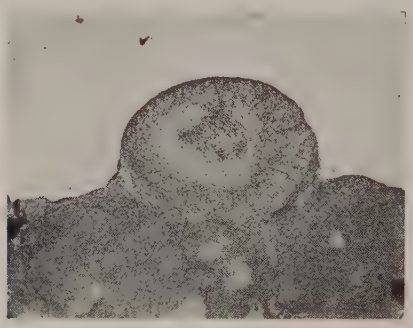


FIG. 1.

Recent corpus luteum, still showing a cavity in its center, in the ovary of a postpubertal hypophysectomized rat (complete removal of the pituitary checked at autopsy), treated with A. P. L. and the ammoniacal pituitary extract.

These experiments show that A.P.L. leads to follicle maturation and corpus luteum formation only in the presence of some pituitary factor which can be replaced in the hypophysectomized animal by the administration of suitable pituitary extracts which in themselves are much less active.

Evans[2] thought that the growth hormone of the pituitary is turned into the maturity hormone by a catalytic action of prolan, whereas Smith[3] reported experiments which induced him to believe that the maturity hormone and A.P.L. are synergistic. Synergism between A.P.L. and the pituitary certainly exists; but we do not feel that it has been definitely proved that the substance which is necessary for the normal action of A.P.L. on the ovary, and which is produced by the pituitary, is identical with any of the known hormones of the hypophysis. As a working hypothesis we simply

[2] Evans, H., *et al.*, *Am. J. Physiol.*, 1932, **100**, 141.

[3] Smith, P. E., reported A. A. A., Section N, Atlantic City, December 28, 1932.

assume, therefore, that a complementary substance is furnished by the pituitary which cooperates with A.P.L. in its effect on the ovary. Whether this substance is identical with any of the known pituitary hormones or not remains to be proved, although experiments now under way in this laboratory seem to indicate that this complementary substance is not identical with the known pituitary hormones.

6623

Automatism of Anuran Lymph Hearts as Obtained by Transplantation.

MARION A. REID. (Introduced by F. H. Pratt.)

From the Department of Physiology, Boston University School of Medicine, and the Bermuda Biological Station for Research, Inc.

Although the normal lymph hearts of Anura are typically neurogenic (Brücke and Umrath[1]) even to being homolaterally synchronized (Pratt and Reid[2]) the beating of these organs *in situ* after interruption of the spinal nerve impulses has often been observed. Such preparations, even if intrinsically ganglion-free, do not altogether eliminate the possibility of some peripheral nervous influence. Moreover, the full, regular beats of excised lymph hearts in isotonic salt solutions originally observed by Moore[3] were not obtained by Brücke,[4] whose results by the same method (irregular contractions like the fibrillations of the blood heart) have since been confirmed by the writer. The method here described, permitting indefinitely continued observation with isolation in a favorable environment, has made possible the recognition and detailed study of a fully developed automatic rhythm.

An anterior lymph heart exposed by the dorsal route is cut away from the surrounding muscles, the hooked transverse process of the third vertebra, and the vertebral vein. The tongue is everted and a small incision made in the basihyoid (retrolingual) membrane near the posterior border of the underlying lymph sac (sinus basi-

[1] Brücke, E. T., and Umrath, K., *Pflüger's Arch.*, 1930, **224**, 631.

[2] Pratt, F. H., and Reid, M. A., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 1019.

[3] Moore, A., *Am. J. Physiol.*, 1901, **5**, 87, 196.

[4] Brücke, E. T., *Pflüger's Arch.*, 1906, **115**, 334.

hyoideus). The extirpated lymph heart, a collapsed oval sac, is inserted through this opening and moved forward to the anterior margin of the sinus, where it usually becomes fixed by the connective tissue. The transparency of the membrane covering this lymph sinus makes it possible to observe the implant at any time by simply everting the tongue.

The observations were made on etherized or spinal animals, usually 10 days or more after operation. In every instance the transplanted tissue had become a firm, spherical mass. In all but 2 of the 13 active cases the tissue was well vascularized by connections with the vessels of the tongue. However, the 2 bloodless organs which floated freely in the lymph of the sinus contracted as spontaneously and coordinately as those that were attached. The rate of beat of the individual transplants varied considerably. A tendency to rapid periodic grouping was characteristic of the younger transplants, but all were eventually capable of long continued regular rhythm.

Not only was the transplanted tissue favorably placed for microscopic observation, but its activity could easily be recorded mechanically or electrically when large frogs *(R. catesbeiana)* or toads *(B. marinus)* were the experimental animals. Kymographic records were made by placing on the transplant a vertical straw supporting the writing-arm of a light heart lever. The responses to artificial stimuli showed that the automatic lymph heart had acquired cardiac-muscular properties. Single shocks produced extra-contractions followed by a pause longer than the normal intersystolic interval; these stimuli were ineffective during systole; a faradic current did not tetanize, although it sometimes increased the rate; and the organ responded maximally at threshold. Curare usually failed to stop an active transplant, while in 2 cases it produced a temporary increase in rate. Adrenalin chloride (1:100,000 and 1:10,000) introduced into the lymph sac had, however, apparently no effect upon either the amplitude or the rate. Throughout the above observations the organ was protected from drying by the thin overlying membrane.

The isolation of the transplants from systemic heart and larger muscle currents is particularly advantageous for electrical recording. By using a liquid-contact lead to an amplifier and bifilar oscillograph an uncomplicated diphasic curve was obtained. The character of this action potential sustains the histological findings that nerve cells are absent from the transplanted tissue, since multiple oscillatory discharges, common alike to the normal lymph heart mechan-

ism[1] and to that of invertebrate hearts (Garrey[3]), seem completely wanting.

The transition to intrinsic automacity on the part of lymphatic hearts is thus shown to involve a radical functional change from skeletal to cardiac muscular properties. Results in detail will appear in a later report.

6624

Studies on the Adrenal. II. Extraction of Cortical Hormone from Urine.

ARTHUR GROLLMAN AND W. M. FIROR.* (Introduced by E. K. Marshall, Jr.)

From the Department of Pharmacology and Experimental Therapeutics and Department of Surgery, Johns Hopkins University.

It is probable from many physiological observations that the active principle of the adrenal cortex is of the nature of a general tissue hormone. If this be the case it should be present, albeit in small quantities, throughout the body and possibly in various secretions. Its presence was therefore sought in the urine.. Perla and Gottesman[1] claimed to have extracted the hormone from urine. From each liter of urine they obtained a quantity of hormone comparable to that obtained from about 225 gm. of the fresh beef adrenal glands by the method of Hartman.[2] Such a high yield would speak either for the excellency of urine as a source of the hormone or for the poor yield obtainable from glands by the method utilized. Unfortunately, Perla and Gottesman used as evidence for the presence of cortical hormone in urine the increased resistance of rats to histamine when the extract was simultaneously injected. The indirect nature of this method of test renders their conclusion open to question.

Our urinary extracts were prepared by extracting the fresh urine with benzene. When other than freshly voided urine was used, the products were less active, having apparently undergone decomposition, as might be readily expected. After washing the benzene with water, it was removed *in vacuo* at 40°C., after adding an

[3] Garrey, W. E., *J. Cell. and Comp. Physiol.*, 1932, **1**, 209.

* Aided by a grant from the Hartley Corporation.

[1] Perla, D., and Gottesman, J. M., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 1024.

[2] Hartman, F. A., *Endocrinol.*, 1930, **14**, 229.

amount of 0.9% saline to make 1 cc. of the final extract correspond to one liter of urine. A yellowish, odoriferous solution was obtained which was capable of prolonging life and permitting growth in one-month-old adrenalectomized rats when administered in doses of a cubic centimeter, twice daily. As compared to the extracts obtained by us from adrenal glands,[3] one liter of urine contains an amount of hormone corresponding to approximately only one-half gram of glandular tissue. It is thus much less valuable as a source of the hormone than indicated by Perla and Gottesman's results.

An attempt was also made to prolong life in adrenalectomized dogs and cats by the urinary extracts. As might be anticipated, the results, although indicating the presence of some activity in the extracts, showed the practical impossibility of preparing sufficient hormone from urine to prolong life of adrenalectomized cats or dogs for any considerable length of time. Thus a dog, bilaterally adrenalectomized at one operation, was kept alive for 9 days. Microscopic examination of the adrenal sites revealed the absence of any residual cortical tissue. In our control series of untreated dogs death resulted on an average on the fourth day with a maximum survival of 7 days (one stage operation in each case). The animal cited received an average daily dose of 17 cc. of extract, which corresponds to the amount of hormone obtained from about 8 gm. of cortical tissue as prepared by our method.[3]

In view of the imputed importance of pregnancy and "heat"[4] in prolonging the life of adrenalectomized animals it was necessary to prove that the above described results were not due to any effect of the sex-stimulating hormones present in urine. Extracts made from the urine of pregnancy were no more potent than those from male urine. Theelin, Antuitrin, and Folluteinâ€ were ineffective in prolonging life of adrenalectomized animals; and hence it is doubtful if the observed results are attributable to the presence of sex hormones. On the other hand, it is necessary to consider the presence of a cortical hormone or its decomposition products in urinary extracts as being possibly responsible for certain effects of such extracts on the reproductive system.

[3] The rat method of assay and the preparation of active cortical extracts from adrenal glands are described elsewhere (Grollman, A., and Firor, W. M., *J. Biol. Chem.*, 1933, in press).

[4] Stewart, G. N., and Rogoff, J. M., *Am. J. Physiol.*, 1927, **79**, 508. Firor, W. M., and Grollman, A., *Ibid*, 1933, **103**, 686.

† We are indebted to Dr. O. Kamm of Parke, Davis and Co., for the Theelin and Antuitrin—S; and to Dr. J. A. Morrell of E. R. Squibb and Sons for the Follutein used in this study.

6625

Host Adaptability of Treponema Pallidum.

E. E. ECKER.*

From the Institute of Pathology, Western Reserve University, Cleveland, Ohio.

Since Bertarelli's discovery[1] that rabbits could be successfully inoculated with the *Treponema pallidum*, this animal has been widely employed in experimental syphilis. Bertarelli noted that takes became more constant after the second transfer of the organism through the rabbit and indicated the possibility of its adaptation to the new host. From then on, the majority of the published studies dealt with the variability of the incubation period, among them an excellent summary by Mulzer.[2] In general, most workers have confined their experimental work to the use of known strains, namely, the Truffi transferred to the rabbit in 1908, the Kuznitzki, the Mulzer, and the Nichols and Hough strains. According to Mulzer the Truffi, also known as the Kolle or Frankfurter strain, causes 100% takes after an incubation period varying from 3 to 4 weeks when injected intrascrotally or intratesticularly. Similarly, the strain of Nichols and Hough has undergone many passages through rabbits since its isolation. Bessemans and Vlaeyen[3] reported that corneal or scrotal scarifications were the least successful routes for the inoculation of rabbits with syphilitic material from man. However, for transplantations from rabbit to rabbit corneal and, above all, scrotal scarifications are the methods of choice. For primary isolations these authors preferred intrascrotal or intratesticular inoculations. This fact definitely indicates the readiness by which a certain tissue-resistance is overcome by a well adapted organism, and to a distinct localized immunity. Zinnser, Hopkins and McBurney also believe that localized immunity is possible and not dependent upon a generalized reaction on the part of tissues remote from the site directly involved in reaction with the invading treponemata.

The *Treponema pallidum* adapted to the rabbit does not lose its virulence for other susceptible animals or man. Uhlenbuth and

* Aided by a grant from the Committee on Research in Syphilis.

1 Bertarelli, E., *Centralbl. f. Bakt. Abt. I*, 1907, **43**, 448.

2 Mulzer, P., *Handbuch der Haut. u. Geschlechtskr.*, **15**, No. 1, p. 290, Berlin, Julius Springer, 1927.

3 Bessemans, A., and Vlaeyen, N., *Arch. Int. de Med. Exp.*, 1928-1929, **4**, 470.

Mulzer[4] have successfully inoculated monkeys, guinea pigs and goats with rabbit syphilitic material, and accidental infections of man from laboratory materials have been numerous considering the relatively few workers in this field.

All of our own (9) strains were isolated in this Institute from primary or secondary lesions of man and their incubation period (in the rabbit) varied from 26 to 84 days. In addition to these new strains we employed (4) older, standard strains, the Nichols and Hough, the Zinsser and Hopkins, obtained through the courtesy of Dr. W. Brown, the Chesney and Hanzlik strains secured through the kindness of Drs. A. M. Chesney and P. J. Hanzlik.

The incubation period of the 13 strains varied from 17 to 99 days. Bessemans and Vlaeyen reported a variation from 18 to 90 days for primary inoculations. For transplantations their periods varied from 32 to 47 days indicating a shortening of incubation periods following transfers.

We have employed a uniform technique, inoculating the animals intratesticularly with about 0.5 cc. of a suspension prepared from an aseptically excised testicle ground up with sterile sand in about 5 to 10 cc. of sterile saline. Massive doses were inoculated and careful attention was paid to avoid all possibility of aspecific infec-

TABLE I.
Comparative Study of Incubation Period and Takes of 2 Groups (New and Old Strains) of the *Treponema pallidum*.

Strain No.	Year	No. inoculated	No. takes	Died before limit of incubation	No. survived for take	Incubation Time			Probable error ±	% takes of living animals
						min.	max.	mean		
I	1929	49	23	8	41	21	60	30.91	1.07	56
II	1930	12	9	2	10	26	46	37.44	1.18	90
*III	1929	5	1	4	1	30	30	30.0	0	100*
* IV	1929	4	0	0	4	—	—	—	—	—*
V	1929	12	6	4	8	33	58	40.5	2.45	75
* VI	1930	7	1	1	5	24	24	24.0	—	20*
VII	1930	25	13	2	23	19	75	38.3	3.11	56
VIII	1930	13	6	2	11	25	78	44.0	4.79	54.5
IX	1929	69	32	25	44	23	99	40.2	1.62	72
Nichols and Hough	1913	29	23	3	26	17	43	26.13	1.10	88.4
Zinsser, Hopkins, Gilbert	1914	23	21	1	22	22	45	31.14	1.06	95.6
Chesney	1924	7	4	2	5	17	25	24.75	0.146	80.0
Hanzlik	1927	24	20	2	22	24	58	33.85	1.84	91.7

* Not considered in series.

[4] Uhlenhuth, P., and Mulzer, P., quoted by P. Mulzer,[2] p. 265.

tions in the normal and infected animals since our problem was concerned with the cultivation of the *Treponema pallidum*. In all, 278 animals were used. The animals were kept in single cages and observed for at least 6 months.

Strains III, IV, and VI, were eliminated from our study because of the small numbers of animals used or the single takes obtained in the small series. Strain IV was extremely difficult to pass through rabbits and eventually was lost. The same is true for Strain VI. Strain VIII showed a large probable error in the group (±4.79) which in all probability is due to a late orchitis developed in one animal at the end of 78 days following inoculation, while the remainder of the series developed the disease in periods varying from 25 to 46 days.

The mean of incubation of the new strains varied from 30 to 44 days, while that of the older strains from 24.75 to 33.85 days. The mean of the maximum incubation time of the older strains almost coincided with the mean of the minimum incubation time for the younger groups. After statistical treatment both groups appeared to be homogeneous within themselves. The significance of the difference in means between the new and the older strains is seen in Table II.

TABLE II.
Significance of Differences Between the Means of the New and Old Strains.

		Mean	Probable Error
New		37.52	±0.98
Old		29.43	±0.83
$\frac{D}{PE_d}$	$= \frac{M_1 - M_2}{PE^2_{M_1} + PE^2_{M_2}}$	$= \frac{37.52 - 29.43}{0.9604 + 0.6889} = \frac{8.09}{1.284}$	$= 6.30$
			%
Incidence of takes by strains NH, ZH, C, and H			90.6
Incidence of takes by strains 1, 5, 7, 8, and 9			62.9
Incidence of takes by strains 1, 2, 5, 7, 8, and 9			64.9

The ratio of difference between means and the probable errors of difference, 6.30, is more than sufficiently large to consider the difference in the means significant, and to warrant a comparison of incidence of takes between the 2 groups. An investigation of the significance of the difference between the group of old strains and the group of new ones, by the method of Fisher,[5] gave 4.03 as the value of which, for the number of degrees of freedom involved, is

[5] Fisher, R. A., Statistical Methods for Research Workers, 1928, p. 107. (2nd edition, Oliver and Boyd, London, Eng.

definitely significant. This confirms the fact that the difference between the mean incubation periods of the 2 groups is real.

The incidence of takes by strains 1, 5, 7, 8, and 9 was 62.9%. Inclusion in this series of the unusually high number of takes in Group II (Table I) raises this figure to 64.9%, while the incidence of the older strains reaches 90.6%. The older strains, therefore, appear to be more consistently infectious for the rabbit than the newer strains.

Conclusions. A comparative study has been made between the infectivity of 2 groups of *Treponema pallidum*, 9 new and 4 older strains. The mean of incubation of the newer group varied from 30 to 44 days, while that of the older group varied from 24.75 to 33.85 days. Statistically, both groups appeared to be homogeneous in themselves and the incidence of takes for the newer strains was 64.9%, while that of the older strains 90.6%, a difference of 25.7%. These findings indicate adaptation of the organism to its new host and should be considered in all work in experimental syphilis.

6626

A Species Limitation of an Enhancing Material Derived from a Mammalian Tumor.

ALBERT E. CASEY.

From the Laboratories of The Rockefeller Institute for Medical Research.

In an effort to differentiate the material derived from the Brown-Pearce rabbit tumor[1] from other enhancing materials it seemed desirable to know whether it would enhance various diseases and tumors, both in the same and in different species. To this end a series of experiments was made with a Bashford mouse carcinoma (No. 63).* The experiments were distributed at approximately 6 weeks intervals between January, 1932, and January, 1933, each experiment consisting of 30 mice divided into 3 groups of 10. Enhancing material prepared from Brown-Pearce tumor tissue[1] and enhancing material prepared in an entirely similar manner from

[1] Casey, A. E., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 816; *J. Exp. Med.*, in preparation.

* The experiments with the Bashford tumor were carried out in the laboratory of Dr. Jas. B. Murphy at his kind invitation. The other experiments on the enhancing material derived from the Brown-Pearce tumor were done in the laboratory of Dr. Wade H. Brown.

the Bashford tumor itself were injected subcutaneously into the left groins of the animals of Groups 1 and 2 respectively. No tumor growths resulted. Twelve to 18 days later the animals of these 2 groups together with those of the third, not previously injected, were inoculated with grafts (Exps. 1-6) or with a saline emulsion (Exps. 7-10) of fresh Bashford tumor tissue (Table I).

TABLE I.
Summary of Data on *Material and Methods.*

Exp. No.	Bashford Preserved Material				Brown-Pearce Preserved Material				Interval between inoculation and reinoculation days
	Age of Tumor Selected days	Temp. Ice-box	Days Preserved	Dose	Temp. Ice-box	Days Preserved	Dose	Mice Strain	
1	20	32°	10	.5	26°	60	.5	Rckf. Inst.	12
2	23	32°	10	.5	—	—	.5	”	12
3	21	32°	10	.5	28°	90	.5	”	12
4	52	32°	28	.5	28°	85	.5	”	13
5	35	32°	40	.5	28°	57	.5	”	12
6	35	32°	40	.5	28°	57	.5	”	12
7	42	24°	21	.3	24°	44	.3	”	15
8	42	24°	35	.3	24°	58	.3	Swiss	15
9	42	24°	50	.1	24°	72	.1	Rckf. Inst.	16
10	42	24°	68	.1	24°	90	.1	Swiss	18

Observations were made on the incidence and the size of the primary tumors resulting. Of the 97 mice treated with the rabbit tumor material 46 (47.4%) had primary tumors at 21 days† as compared with 57 (57.5%) among the 99 control mice and 67 (67%) among the 100 mice treated with material from the Bashford tumor itself. Such an excessive variability would not be expected to occur by accident more often than twice in 100 similar experiments with 300 mice (x^2—7.7, n—2, P—0.02). Treatment of the mice with the rabbit tumor material seemed to result in fewer takes, while treatment of the mice with the mouse tumor material seemed to result in an increased number of primary tumors, the difference between the 2 series being significant when compared directly (x^2—7.7, n—1, P—0.01—).

The mean size of the 46 primary tumors among the mice treated with the rabbit tumor material was 1.77 cc. and that of the 57 tumors among the controls 1.54 cc., while that of the 67 tumors among the mice treated with the homologous mouse material was 3.16 cc. There was no significant difference between the size of the primary tumors of the first 2 groups (s_d—0.29, t—0.79, P—0.42) but a very significant difference between each of these and the size

† The observations at 21 days were selected from the weekly observations available because of the frequency of deaths after this period.

TABLE II.
Summary of Results by Groups.

Exp. No.	Incidence of Primary Tumors			Vol. Primary Tumor in Mice with Tumor			Vol. Primary Tumor per Animal Inoculated		
	B.P.XYZ	Cont.	Bash.XYZ	B.P.XYZ	Cont.	Bash.XYZ	B.P.XYZ	Cont.	Bash.XYZ
	%	%	%	cc.	cc.	cc.	cc.	cc.	cc.
1	44	78	50	1.0	1.2	3.8	0.5	0.9	1.9
2	10	10	30	4.6	0.4	0.2	0.5	0.0	0.1
3	30	60	80	0.9	1.7	2.1	0.3	1.0	1.7
4	50	70	90	1.0	1.3	5.1	0.5	0.9	4.6
5	50	80	90	1.7	1.0	2.2	0.9	0.8	2.0
6	30	30	50	0.6	0.7	1.4	0.2	0.2	0.7
7	70	60	60	1.7	2.0	2.6	1.2	1.2	1.5
8	90	80	90	3.0	3.1	5.9	2.7	2.5	5.3
9	40	30	30	1.8	0.6	4.3	0.7	0.1	1.3
10	63	80	100	1.7	1.4	2.0	1.1	1.1	2.0
Mean for Groups	47.7	57.8	67.0	1.8	1.3	3.0	0.8	0.9	2.1
Mean for Individuals	47.4	57.5	67.0	1.8	1.5	3.2	0.8	0.9	2.1

B.P.XYZ—Mice treated 2 weeks previous to the regular inoculation with fresh Bashford tumor with enhancing material derived from the Brown-Pearce tumor.

Controls—Mice inoculated with fresh Bashford tumor only.

Bash.XYZ—Mice treated with enhancing material derived from the Bashford tumor itself 2 weeks previous to the regular inoculation with fresh Bashford tumor.

of the primary tumor among the mice treated with the homologous mouse tumor material (s_d—0.37, 0.34, t—3.8, 4.8, P—0.01—). The enhancing material from the Brown-Pearce rabbit tumor, therefore, possibly diminished the incidence but had no significant effect on the size of the Bashford primary tumor while the enhancing material obtained from the same Bashford tumor enhanced both the incidence and the size of the primary tumors, the mean size of the primary tumors being twice that of the controls. Postmortem examination of mice found dead and of surviving mice 30-55 days after inoculation revealed no metastases visible to the unaided eye.

The conclusions to be drawn are that a rabbit tumor material which greatly enhanced both the primary and the metastatic phases of the same tumor of the rabbit (the Brown-Pearce tumor) failed to enhance either the primary or the metastatic phase of a Bashford carcinoma (No. 63) of the mouse. Material derived from this latter, however, had a noteworthy enhancing effect on growths of the same sort.‡ It should be remarked that the enhancing material

‡ That this Bashford tumor contains an enhancing material which enhances both the incidence and the size of the primary tumor (no mention was made of metastases) confirms the findings of Haaland (*Lancet*, 1910, **1**, 787) and of Leitch (*Lancet*, 1910, **1**, 991).

from the Brown-Pearce tumor, a growth which normally produces metastases, enhanced both the incidence and the development of metastases in this disease, and that the enhancing material from the Bashford tumor which ordinarily does not produce metastases did not cause the occurrence of metastases recognizable in the gross. The enhancing materials derived from two mammalian tumors seemed to conform to type and one at least is limited in its activity.

6627

Traumatic Shock in Adrenalectomized Rats.

S. C. FREED. (Introduced by H. M. Evans.)

From the Institute of Experimental Biology, University of California.

It is well known that an adrenalectomized animal is susceptible to a much smaller dose of a poison than a normal animal. The drugs which have been used in this work are many and quite unrelated. They include histamine (Dale[1]), morphine and bacteria (Scott[2]), and many others (Lewis[3]). Wyman and Tum Suden,[4] however, feel that histamine is fatal in small doses because of absence of the medulla and that intraperitoneal injections of epinephrine can protect against fatal doses of histamine. Nevertheless, cortical extracts are much more effective than epinephrine in this rôle (Marmorston-Gottesman and Perla[5]). Likewise cortical extracts are capable of protection in adrenalectomized animals against fatal doses of typhoid vaccine (Scott and Bradford,[6] Perla and Marmorston-Gottesman[5]).

The sensitivity of adrenalectomized animals to histamine seems a very significant relationship inasmuch as many common serious clinical conditions are assumed to be the result of histamine poisoning. It has been generally accepted from the work of Cannon,[7]

[1] Dale, H. H., *Brit. J. Exp. Path.*, 1920, **1**, 103.

[2] Scott, W. J. M., *J. Exp. Med.*, 1923, **38**, 543; 1924, **39**, 457.

[3] Lewis, J. T., *Am. J. Phys.*, 1923, **64**, 506.

[4] Wyman, L. C., Tum Suden, C., *Am. J. Phys.*, 1932, **99**, 285.

[5] Marmorston-Gottesman, J., and Perla, D., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 1022.

[6] Scott, W. J., and Bradford, W. L., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 428.

[7] Cannon, W. B., *Compte rend. Soc. Biol.*, 1918, **81**, 850.

Bayliss,[8] and Dale and Laidlaw[9] that traumatic shock is the result of absorption of histamine-like substances from injured tissue into the blood giving the typical histamine reaction, fall in blood pressure, asthenia and sub-normal temperature. These symptoms can be explained on the basis of altered capillary permeability, allowing the escape of plasma into the tissues with the subsequent dehydration of blood.

Rats are very resistant to histamine requiring about 800-1000 mg. per kilo to cause death. It is well known, too, that it is impossible to shock a rat by trauma. Many rats in this laboratory have had all their leg muscles crushed and after recovery from ether anesthesia, return to normal activity except for limping. Because of the loss of resistance to histamine by removing the adrenals we wished to see whether there would be an analogous susceptibility to injured tissues.

Young, vigorous, male rats 35-40 days old were bilaterally adrenalectomized. They were allowed 36 hours to recover from the operation. They were, then, anesthetized with ether and their hind leg muscles crushed with long-nosed pliers. Normal rats were treated likewise and other adrenalectomized rats were simply anesthetized.

The controls recovered completely. For some time after the bruising treatment the adrenalectomized animals appeared normal but within an hour they all began to show unmistakable symptoms of shock. They crouched in corners with fur erect, obviously sick. At the end of 2 hours they were in deep shock, cold, cyanotic and very feeble. One rat survived, 7 rats died at the end of 1½, 3, 4. 5, 6, 7, and 12 hours.

The one that survived began to recover after 6 to 7 hours but it was not normal until about 18 hours from the beginning of the experiment.

A second group of adrenalectomized rats were injured similarly, 18 hours after their adrenals had been removed. Some were injected subcutaneously with 5 cc. normal saline at the time of injury and a second 5 cc. two hours later as they lay in shock. The addition of fluids did not prevent the progress of severe shock, but it did prevent death in most cases. Recovery was apparent after 4-5 hours of complete collapse. Seven of the 9 rats which received no fluids died at the end of 2, 2½, 4, 5, 6, 9, and 10 hours. Two sur-

[8] Bayliss, W. M., Oliver Sharpey Lectures, 1918.

[9] Dale, H. H., and Laidlaw, P. P., *J. Phys.*, 1918, **52**, 355.

vived. Of the 9 rats receiving the fluids one died at the end of 10 hours, 8 survived, but eventually they died of adrenal insufficiency about 9 days later.

The work of Cannon and of Bayliss has long been undisputed. Their theory that shock is due to the absorption of histamine is now being questioned. Robinson and Parsons[10] claim that secondary shock can satisfactorily be explained by the loss of blood into the injured tissues. Blalock *et al.*[11] find that enough fluids escape into the injured and neighboring parts to account for the circulatory collapse. In a recent review on the etiology of traumatic shock (Rikstinat[12]), the loss of blood and fluids into the site of injury is the favored theory. We offer in our experiment some facts which require an explanation other than this.

Our normal rats had extensive tissue damage without the formation of shock. If, however, the adrenals were previously removed, trauma to much less tissue was sufficient to produce definite and usually fatal shock. The swelling of the tissues was no greater in the latter group and therefore loss of fluids into injured tissues could not account for this condition. The only conclusion to be drawn is that the dehydration in the adrenalectomized rats was due to loss of fluids into the general body tissues, a typical histamine reaction. We can nicely apply the theory of Cannon, Bayliss and Dale here. The amount of histamine liberated by the trauma was ineffective in normal rats, but was potent in the sensitive adrenalectomized rats.

Summary. 1. Adrenalectomized, but not normal rats are sensitive to trauma and usually die from moderate tissue injury. 2. Injection of normal saline is effective in protecting shocked rats from death. 3. The toxemia theory of shock explains this susceptibility of adrenalectomized rats to injury.

The writer would like to express his appreciation to Professor Herbert M. Evans for his encouragement and aid in the prosecution of the research.

[10] Robinson, W., and Parsons, E., *Arch. Path.*, 1931, **12**, 1.

[11] Blalock, A., *Arch. Surg.*, 1930, **20**, 959; Blalock, A., Beard, J. M., and Johnson, G. S., *J. Am. Med. Assn.*, 1931, **97**, 1794.

[12] Rikstinat, G. J., *Arch. Path.*, 1932, **14**, 378.

6628

Thyreotropic Hormone of Anterior Pituitary.

E. M. ANDERSON AND J. B. COLLIP.

From the Department of Biochemistry, McGill University, Montreal, Canada.

A number of investigators have reported the production of hyperplasia of the thyroid in the guinea pig by the injection of anterior pituitary extracts (Loeb and Bassett,[1] Janssen and Loeser,[2] Junkmann and Schoeller,[3] Aron[4]). Loeb and Aron have reported failure to obtain this effect in the albino rat. We have previously reported the production of hyperplasia of the thyroid of the rat with a marked hyperthyroidism by means of a crude alkaline extract of anterior pituitary and a killed staphylococcus culture.[5]

We have recently prepared a highly purified extract of the anterior pituitary containing the thyreotropic factor. This has been obtained from the residues after the removal of the growth hormone. It has been freed of prolactin by isoelectric precipitation of this fraction, and further purified by salt precipitation and fractionation by alcohol and acetone. Assayed by the Junkmann and Schoeller method 0.3 mg. total solids contains one unit.

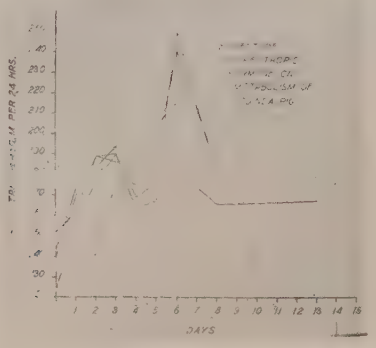


CHART 1.

Showing the rise in metabolic rate of guinea pigs receiving daily injections of the thyreotropic hormone.

[1] Loeb and Bassett, PROC. SOC. EXP. BIOL. AND MED., 1929, **26**, 860.

[2] Janssen and Loeser, *Arch. f. exp. Path. u. Pharmakol.*, 1932, **163**, 517.

[3] Junkmann and Schoeller, *Klin. Wchnschr.*, 1932, **11**, 1176.

[4] Aron, *Rev. Française d'Endocrinologie*, 1930, **8**, 472.

[5] Anderson, *Canad. Med. Assn. J.*, 1933, **28**, 23.

Chart 1 shows the rise in metabolic rate of 5 guinea pigs given daily injections of the thyreotropic extract. These are representa-

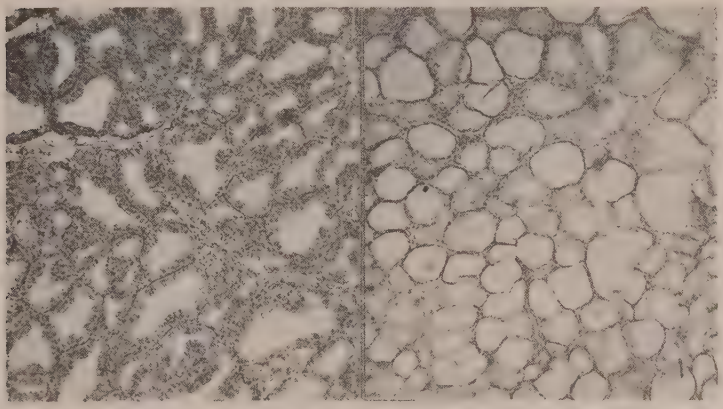


FIG. 1 A. FIG. 1 B.

A. Shows marked hyperplasia of the thyroid of a guinea pig receiving the thyreotropic hormone. Animal was killed when metabolic rate was increased 50%.
B. shows the thyroid of an untreated guinea pig.

tive curves of a series of 12 animals. Those animals which were killed when the metabolic rate was elevated showed hyperplasia of the thyroid (Fig. 1).

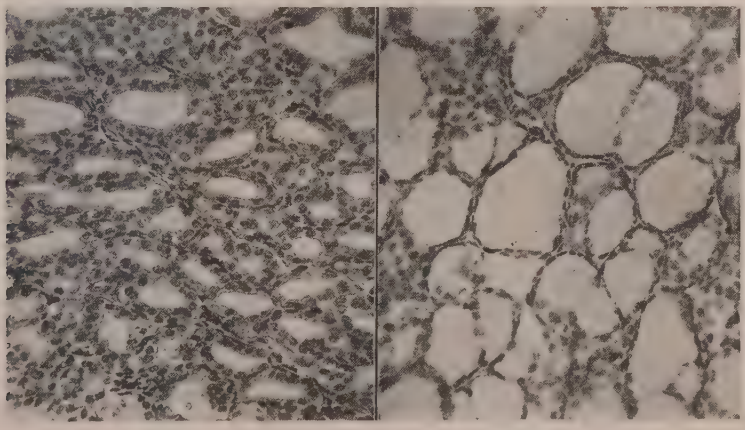


FIG. 2 A. FIG. 2 B.

A. shows the thyroid of an hypophysectomized rat after injections of 2 cc. daily of the thyreotropic extract for 8 days.
B. shows the thyroid of a control rat of this series.

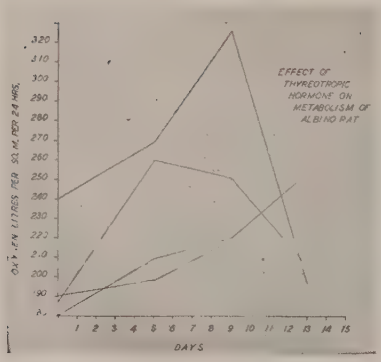


CHART 2.

Showing the rise in metabolic rate of rats receiving daily injections of the thyreotropic hormone.

A purified thyreotropic extract administered to a group of 8 hypophysectomized rats prevented the atrophy of the thyroid which invariably occurs in the untreated hypophysectomized animal (Fig. 2).

Chart 2 shows the effect of a thyreotropic extract on the metabolic

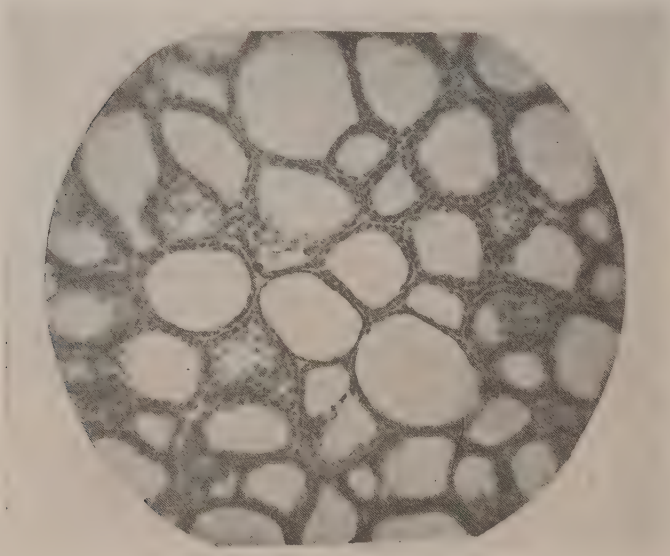


FIG. 3.

Shows the thyroid of a rat receiving 4 cc. daily of the thyreotropic extract for 13 days, during which time the metabolic rate had been increased 50% and then returned to normal. Some of the alveoli show an atrophic type of epithelium.

rate of 4 rats. The thyroid from one of the rats killed on the ninth day of injection showed a marked hyperplasia. The thyroid glands of the 3 rats which were treated for 13 days showed an unusual picture. In 2 cases there was a generalized hyperplasia, but scattered throughout the gland were large alveoli with a flat, atrophic type of epithelium. The thyroid of the third rat is shown in Fig. 3. Many of the alveoli have a normal epithelium, while others show an atrophic type.

Summary. We have reported the physiological effects of a highly purified extract of the anterior pituitary containing the thyreotropic hormone, which gives complete replacement therapy in the hypophysectomized rat and also produces a hyperplasia of the thyroid with hyperthyroidism in both the rat and the guinea pig.

6629

Tobacco Sensitiveness in Angina Pectoris and Coronary Artery Disease.

JOSEPH HARKAVY. (Introduced by George Baehr.)

From the Out-Patient Department and Laboratories, Mount Sinai Hospital, New York.

Harkavy, Hebald and Silbert[1] reported the incidence of tobacco hypersensitiveness in 68 cases of thrombo-angiitis and in 122 controls. In this study it was found that 83% of thrombo-angiitis cases were hypersensitive to various tobacco extracts when tested by the intradermal method. Thirteen out of 20 patients were demonstrated by the passive transfer method to have reagins for tobacco. Only 10% of the control smokers reacted to tobacco.

The present investigation deals with results of a similar study in patients with arteriosclerosis of the coronary arteries who presented the clinical syndrome of angina pectoris, with diagnostic electrocardiographic changes. The tobacco employed in these tests was separate extracts of Burley, Maryland, Virginia, and Xanthis (Turkish tobacco), prepared according to the method of Coca. An extract consisting of a mixture of tobaccos obtained from the Allergy Department of the New York Hospital through the courtesy of Dr. Cook was also employed.

Of 36 patients with coronary artery disease, all of whom were

[1] Harkavy, Hebald and Silbert, PROC. SOC. EXP. BIOL. AND MED., 1932, **30**, 104.

smokers, 13 or 36% were found to give positive intradermal reactions of the urticarial type to one or another tobacco. Two cases were also tested with extracts of tobacco smoke. These tests were carried out with a saline extract (Coca) of a concentrate, prepared by Dr. Harry Sobotka from an alcoholic solution of cigarette smoke. Both patients gave positive intradermal reactions. A final conclusion, however as to the significance of smoke reactions will have to be reserved. The average age of the 13 patients who reacted positively to tobacco extracts was 45. Four of these had a personal history of allergy. The average age of the 23 patients who did not react to tobacco was 60 years.

The serum of 6 of the positively reacting patients was studied for the presence of specific reagins to tobacco by the passive transfer method of Prausnitz and Kustner. In 4, reagins could be demonstrated.

The extract of mixed tobacco obtained from Dr. Coca which was nearly free of nicotine gave the largest number of positive reactions. Twelve patients tested with 10% solution of nicotine tartrate 1:10,000 and 1:5,000 were found to be negative.

6630

Anatomy of Normal and Reduplicated Limbs in Urodeles.

ISABEL W. HARPER. (Introduced by R. G. Harrison.)

From the Osborn Zoological Laboratory, Yale University.

A study of the anatomy of double limbs in urodeles with especial reference to musculature and blood vessels was made as a basis for a further understanding of the process of reduplication.

Double limbs were produced by rotation of the fore limb bud, both in the normal position and 3-5 segments posterior, in a number of species of Amblystoma (Ambystoma) and in *Eurycea bislineata*. Statistical treatment revealed specific differences in the proportions of total suppression, of reduplication and the subsequent resorption of one member of the pair. More cases of reduplication occurred in those species in which the growth of the limbs is most rapid. Most frequent resorption of one member, however, was found in those with the fastest general growth. *A. tigrinum* showed the greatest number of cases of total suppression. Limbs transplanted

to the flank were more often completely suppressed than were the ones rotated in the orthotopic position, but among the reduplications fewer members were resorbed.

The musculature of double limbs was found to be reduplicated from a point slightly proximal to the junction of the members. While distally the muscles of double limbs in the orthotopic position were entirely similar to those of the heterotropic limbs, the shoulder muscles of these series differed in 2 ways. First, the intrinsic muscles were found in the heterotopic limbs only if the parts of the girdle to which they are normally attached were present, and since only 3½-somite grafts were taken, the heterotopic girdles were often mere plates representing just the central portion of the girdle. Second, the 4 extrinsic muscles which are derived from the myotomes and the gill musculature were never found in the heterotopic limbs, although the latissimus dorsi and the pectoralis, which develop from the limb itself, were usually present.

The normal development of the circulatory pattern in the fore limb was essentially the same in all the species examined. It was worked out in both living and injected specimens, and the results compared with those of Hochstetter[1] and Grodzinski.[2]

The blood vessels of the members of a reduplicating limb join to form a common artery and vein. Circulation begins late and is more subject to interruption than in normal limbs because of the smaller caliber of the vessels. It is, however, established relatively somewhat earlier in harmonic than in disharmonic members (those with the laterality of the other side of the body). The pattern and the direction of flow in the blood vessels of any member are in accordance with its laterality. The minimum circulation for each digit is one vascular loop. If stasis occurs in a digit, and the circulation does not become reestablished, the digit is resorbed. On the other hand, stasis may be prolonged and yet the circulation finally resumed.

Lack of circulation cannot always be the cause of resorption, since when disharmonic members are undergoing resorption they retain at least one vascular loop even when reduced to mere spurs.

When reduplications on the flank were compared with those in the normal position it was found that with respect to the time at which the circulation appears and in the development of the pattern they agree entirely. The blood supply of these heterotopic

[1] Hochstetter, F., *Morph. Jahrb.*, 1891, **17**, 1.

[2] Grodzinski, Z., *Bull. de l'Acad. Polonaise des Sci. et des Lettres, Classe des Sci. Math. et Nat., Serie B, Sciences Naturelles* (II), 1930, 247.

limbs comes from one of the segmental branches of the dorsal aorta, and returns through the renal portal system, the postcaval or posterior cardinal vein.

During the early stages of the growth of the limb the development of the circulatory pattern is correlated with that of the skeleton, proceeding most rapidly in the distal region, at a time when the muscles are differentiating in the proximal part of the limb. The collateral vessels and the details of the proximal region appear after the formation of the joints and at the time of the beginning of function.

Since the development of the vascular pattern follows rather than anticipates the laying down of the skeleton in a reduplicating limb, it would appear that an abnormal circulatory pattern is not one of the causes of reduplication.

6631

Effects of Rate of Growth on Post-natal Development of the White Rat.

G. B. MOMENT. (Introduced by R. G. Harrison.)

From the Osborn Zoological Laboratory, Yale University.

In order to investigate the reactions of the various organs of the body to a radical acceleration in growth rate, over 200 male albino rats of a homogeneous pedigreed strain were fed in such a way that one-half of them grew approximately twice as fast as the other half. This increase in rate of growth was effected by adding greater amounts of yeast and lettuce to an already adequate diet, hence the "slow" growth animals were not stunted but themselves grew somewhat faster than the standard given by Donaldson. At body weights of 60, 90, 120, 150, 180, 240, 300, 360, 420 and 480 gm., 10 rapid and 10 slow growth rats were killed and studied.

A comparison of the 2 groups of rats gives the following results: (1) The ratio between body length and body weight is the same at any given length or weight for both the rapid and slow growth rats and also for Donaldson's animals. (2) The size (measured in terms of wet weight) of 3 different muscles, of the kidney, spleen, thyroid and pituitary depends upon the size of the rat and not upon its age. The same is true, in the main, of the heart and liver, but there is a definite tendency for the rapidly grown rats to have larger

livers and hearts than the slowly grown ones of the same body weight. (3) The size of the eyeballs is a function of age rather than body size, furthermore the growth of the eyes in both rapid and slow growth rats is "heterogonic" and differently so in each case. Rats grown rapidly up to adult size and then maintained at that point until as old as slow growth rats that have just reached full stature have eyes as large as those of the slow growth animals. In other words, the eyes have continued to grow while the rest of the animal was constant. (4) The thymus in rapidly growing rats is enormously larger than in slowly growing ones of the same body weight, attaining a maximum 2 or 3 times the maximum for the slow growth rats. But the age at which the maximum size and involution of the thymus take place is the same in both classes of rats even though their body sizes are very different. (5) The organs of the rapidly grown animals have a water content that is, within the limits of experimental error, like that of the slowly grown ones. Variability in the size of organs was not appreciably increased by the increased growth rate. (6) The final retardation in rate of increase, *i. e.*, the definitive size of all the organs studied except the eyes and the thymus, seems to be determined not by the chronological age of the animal but by its position on the growth curve. At a body weight of about 420 gm. some growth inhibiting mechanism seems to come into play, being especially noticeable in the case of the musculature.

The 3 divergent reactions exemplified by the muscles, thymus and eyes may be explained in part at least on the theory that at different nutritional levels the partition coefficients (measuring the relative distribution of food materials between the various organs) become different.